Nuclear Weapons Effects Research No. 03.011

Sub-task Title: The Effect of Total Body Irradiation on Immunologic Tolerance of Bone Marrow and Homografts of Other Living Tissue

Service Project No.: DA-49-146-XZ-032
Modification No. 3

4.5-P # 4.60

Directing Agency: DASA

Contractor: Baylor University College of Medicine
Houston, Texas

Project Officer: CDR R. L. Gade
Biomedical Division
Headquarters, DASA
Washington 25, D.C.

Project Coordinator and
Principal Investigator: Vincent P. Collins, M.D.
Professor of Radiology
Baylor University College of Medicine
Houston, Texas

Classification: Unclassified

Report Period: Feb. 1, 1963 - Jan. 1, 1964
Final Report

Investigations in the effect of total body irradiation have been carried out in this department for more than 10 years and this final report is based on data collected during this time. The project was initiated when comparison of response of patients receiving therapeutic total body irradiation with that of patients receiving chemotherapy indicated that, in the dose range used, total body raidation was well tolerated and could be used as as adjunct to treatment of disseminated cancer (1, 2). Intensive studies on response of erythropoiesis were undertaken (3) and findings gave new directions for further study so that, in the final years, the protocol included a large series of clinical, hematologic, biochemical and physiological tests (4) as well as studies in protection. The investigation includes observations on 112 patients receiving therapeutic total body irradiation; pilot and parallel studies were carried out on experimental animals.

The fundamental problem has been to define effect of irradiation and quantitate effect with amount of radiation exposure. Since quantitation is possible only to the degree that measurement of both effect and amount of radiation is possible, it was necessary to consider all the variables that would influence measurement.

Comprehension of the problem begins with the recognition that a simple numerical expression cannot define dose of irradiation. The physical characteristics of irradiation require that dose be described not only by the number of units (roentgens, rads, etc.) but also by distribution and intensity in a given volume of tissue, and time or duration of exposure. For each irradiation procedure planned, dosimetry studies were carried out so that uniform distribution of irradiation could be achieved. Several methods, and types of dosimeters, were used for comparison and for accurate measurement of dose to specific anatomical sites. These methods assured even distribution of radiation and produced comparable data on radiation exposure throughout the entire study.

Similar dosimetry studies were carried out for animal experiments. Protocols required animals of similar age, weight and physical structure so that volume irradiated and response could be compared. All animals received immunization vaccine, were quarantined for two weeks, and all were well nourished and in good health prior to experimental work.

Although variables associated with patients requiring therapeutic total body irradiation are present, certain aspects were comparable. All patients were adults suffering from disseminated cancer, all received evenly distributed exposures of irradiation and, except when reaction was severe, none received supportive treatment that would mask effect of irradiation. Since prediction of effect is the basis of radiotherapy, experience in this field is helpful in evaluating response to treatment and in estimating response to heavy exposures by extrapolation of data obtained with therapeutic exposures.

Therapeutic Total Body Irradiation

Examinations and laboratory tests were done on all patients prior to therapeutic total body irradiation to obtain a base line for comparison with tests made during and following treatment. Records of all clinical findings, results of all tests, factors, technique and dose of irradiation were maintained. Follow-up continued as long as the patient survived and, when possible, autopsy was obtained to determine whether or not there was evidence of radiation change. These records formed the basis for analyses of changes by various parameters as presented in annual progress reports.

The patients were divided into 3 groups according to duration (elapsed time) of radiation exposure. These are defined as: I - Single Exposure, II - Protracted Irradiation (given within a period of 2 months), and III - Repeated courses (period ranging from 3 months to 3½ years). Because tolerance to radiation increases with protraction of time, there is a tendency to emphasize protraction while overlooking the amount that can be given safely in a single exposure. When nearing the terminal stage of disease, few patients are likely to survive a protracted course of therapy and dose, which is necessarily low for each treatment of a protracted course, is not likely to prove beneficial in the brief time available for therapeutic effect. Thus, the majority of patients (71/112 - 63%) in this series received single exposures. It seemed possible that response in this group might be compared with data obtained from study of accident victims and exposed Japanese populations who received single exposures.

Group II is composed of 34 (30%) patients receiving protracted irradiation. These were selected because of generalized disease that might be expected to respond to small repeated exposures. It was expected that study of this group would provide information concerning response that might be useful among military personnel when occupying a radioactive area.

The third group is composed of 7 patients who had received either single or protracted exposures and who, after months or years of remission of disease, developed recurrent symptoms requiring further treatment by total body irradiation. These 7 patients received a total of 16 separate courses of treatment or 9 studies in addition to the 112 patients. The number of patients, amount and range of irradiation exposure, and elapsed time, is summarized in Table I.

Table I

Category	Patients	Range of Exposure and Time
I.	71 (63%)	25r - 250r/1 day
II.	34 (31%)	25r/4 days - 400r/32 days
III.	7 (6%)	50r-250r/5 mos. - 2 courses 50r-200r/3½ yrs. - 4 courses

Table 2 summarizes the number of patients receiving total body irradiation in a single exposure. Amount of exposure ranged from 25r to 250r, depending upon the therapeutic indications for treatment.

Table 2

No. of Patients	Total Body Irradiation (single exposure)
6	25r
20	50r
5	75r
8	100r
1	125r
7	150r
22	200r
1	215r
1	250r

Table 3 summarizes the number of patients receiving therapeutic total body irradiation, protracted over periods ranging from 2 to 33 days.

Table 3

No. of Patients	Amount of Irradiation	Elapsed Time
1	25r	4 days
2	50r	2 - 5 days
2	100r	7 - 28 days
9	150r	3 - 18 days
1	185r	7 days
4	200r	6 - 19 days
1	215r	21 days
6	250r	7 - 17 days
4	300r	6 - 33 days
2	350r	22 days
1	400r	23 days
1	545r	18 days

Table 4 outlines the amount of radiation with duration of exposure and time lapse between each course for the 7 patients receiving repeated courses of therapeutic total body irradiation.

Table 4

Patients	Time Lapse	Total Time	Amount of Radiation
M.M.	0		125r/62 days
	8 mos.		50r/11 days
	17 mos.	25 mos.	282r/113 days
F.M.	0		200r/1 day
	5 mos.	5 mos.	200r/1 day
I.S.	0		90r/35 days
	3 mos.		30r/16 days
	1 mo.	4 mos.	50r/63 days
E.R.	0		100r/35 days
	4 mos.		50r/14 days
	9 mos.		150r/1 day
	29 mos.	42 mos.	200r/9 days
M.B.	0		150r/1 day
	10 mos.		100r/1 day
	7 mos.	17 mos.	50r/1 day
J.B.	0		250r/58 days
	5 mos.	5 mos.	50r/1 day
J.T.	0		100r/7 days
	25 mos.	25 mos.	100r/6 days

Systemic reaction following therapeutic total body irradiation is difficult to evaluate because of the influence of disease, medication, and psychogenic factors. Malaise following irradiation was recognized and reported as early as 1897 but the train of symptoms, in 1912 termed "Rontgenkater" (roentgen-hangover), for a given amount of radiation varies widely. Ellinger summarized a series of biochemical changes (5) which he believed accounted for "radiation sickness." Since symptoms may appear following exposures too light to produce detectable biochemical changes, and may not develop following marked changes, other factors must be considered.

For evaluation of the present series, symptoms were graded according to severity: 0 = no symptoms, 1 = anorexia only, 2 = nausea only, 3 = nausea and vomiting one day, and 4 = nausea and vomiting more than one day. Two patients are excluded from this study; one, terminal at the time of treatment, died within 24 hours, and one, an out-patient with concurrent tuberculosis, was lost to follow-up. Table 5 summarizes the severity of symptoms according to single, protracted and repeated courses of exposure among 110 patients.

Table 5

Radiation Exposure	No. of Patients	Symptoms (grades) 0	1	2	3	4
I-Single exposure	69	54	4	3*	8*	0
II-Protracted exposures	34	29	2	2	1	0
III-Repeated courses	7	6	0	0	0	1
Total	110	89	6	5	9	1

*One patient with Grade 2 and one patient with Grade 3 symptoms had nausea and vomiting prior to irradiation.

A total of 21 patients (19%) developed reactions varying from mild to severe but this cannot be accepted as an indication of incidence that might occur in an exposed population. In this group any or all symptoms could be due to disease (2 patients had symptoms prior to treatment), but there is no clue to explain why 89 patients (81%) developed no symptoms at all.

In evaluating radiation sickness according to amount of irradiation, patients receiving single exposures are studied because response is easily recognized and is not complicated by cumulative effect of repeated exposures. Table 6 shows the reaction of 69 patients receiving single exposures of therapeutic total body irradiation

Table 6

Amount of Irradiation	No. of Patients	Symptoms (grades) 0	1	2	3	4
25r	5	5	0	0	0	0
50r	20	15	0	2	3	0
75r	5	5	0	0	0	0
100r	8	8	0	0	0	0
125r	1	1	0	0	0	0
150r	7	5	2	0	0	0
200r	21	15	2	1	3*	0
215r	1	0	0	0	1	0
250r	1	0	0	0	1*	0
Total	69	54	4	3	8	0

*One patient receiving 200r and one receiving 250r had nausea and vomiting prior to irradiation.

Study of the table shows the incidence of symptomatic response to total body irradiation is practically the same for patients receiving 50r as for those receiving 200r, but 14 patients receiving 75 to 125r failed to develop complaints. Review of the clinical histories of the 5 patients with symptoms following 50r reveals that 4 were occupants of the same ward at the same time. The subject of radiation sickness had been publicized in the popular press, was discussed by all occupants of the

ward and anticipated by the 4 scheduled for treatment. The 5th patient, terminal at the time of treatment, was receiving heavy doses of narcotics for pain from widespread involvement of disease from primary breast cancer; in this case, symptoms could have been caused by disease, medication, motion and manipulation, or a combination of these factors.

A careful study of all patients who developed symptoms following therapeutic total body irradiation indicates that, for levels up to 200r in single or protracted exposures, radiation sickness may be avoided by proper management. The attitude and experience of professional personnel, the intentions of personal acquaintances in contact with the patient, the reliability of information in the popular press, and the stability of the patient,--all these enter into the problem of motivation or suggestion. It is probable that, with correct information and proper preparation, normal healthy individuals could tolerate even higher exposures without undue incapacitation.

The goal of treatment by total body irradiation for patients with disseminated cancer is relief of symptoms. Depsite the fact that many patients were approaching the terminal stage when referred for treatment, therapeutic response was evident in many by decrease in size of nodes, relief of impending obstruction and by decreased narcotic requirements subsequent to relief of pain. In some instances, response was dramatic; a few completely bedridden patients became ambulatory and several experienced long term remissions. Among the 112 patients there were more than 30 different types of cancer; since total body irradiation was given only for generalized disease, a survival of one year or even temporary remission was gratifying. In Table 7, the diagnoses are shown in 17 related groups with the number of patients in each group.

Table 7

Diagnosis	No. of Patients
Leukemia (chronic & acute)	14
Lymphosarcoma	23
Lung cancer	12
Hodgkin's disease	6
Multiple myeloma	9
Head & Neck (larynx, tongue, etc.)	7
Testicular cancer	5
Breast cancer	3
G.I. tract (stomach, pancreas)	4
Gynecological cancer	2
Mycosis fungoides	2
Ewing's sarcoma	2
Carcinomatosis (primary unknown)	5
Miscellaneous cancer (paraganglioma, cancer of liver, kidney, prostate, & malignant melanoma)	14
Total	112

Among patients with far advanced cancer, effect of treatment cannot be measured in terms of survival but survivals, through the expected period of depression and recovery, provide information on immediate biologic and physiologic response to total body irradiation. Table 8 summarizes the survival of all patients in the study.

Table 8

Survival Time	Number of Patients		
	Died	Lost to F.U.	Living
0 - 3 mos.	47	6	0
3 - 6 mo.	21	0	0
6 - 12 mos.	10	0	0
1 - 2 yrs.	11	0	0
2 - 3 yrs.	4	0	0
3 - 4 yrs.	0	1	1
4 - 5 yrs.	0	0	0
5 - 6 yrs.	0	1	1
6 - 7 yrs.	1	2	0
7 - 8 yrs.	1	1	3
8 - 9 yrs.	0	0	0
9 - 10 yrs.	0	0	0
10 - 11 yrs.	0	0	2
	95	10	7

Of the 95 patients known to have died, 93 died of disease within 3 years following treatment. Ten patients (9%) were lost to follow-up but 4 of these were observed for periods ranging from 3 to 7 hears before they were lost. Seven patients are living with survival ranging from 3 to 10½ years.

Although patients with disseminated disease from many types of cancer received therapeutic total body irradiation (see Table 7), greatest benefit is derived by those with lymphoma, manifested chiefly in enlarged lymph nodes without marked bone marrow involvement. Over the past 10 years, the increasing activities of the Chemotherapeutic Trials Committee have reduced the number of referrals of such patients for radiotherapy to practically zero. Thus, the majority of patients are those who failed to respond to chemotherapy or those who were refused chemotherapy because of anemia and poor general condition. Among cancer patients, anemia may be caused by infiltration of cancer cells in bone marrow with replacement of normal pre-cursor cells and by shortened red cell survival (6). With widespread cancer, anemia, whether frank or subclinical, is present and death within a short time is inevitable. When patients are referred as a "last resort," the radiotherapist does not wish to withhold treatment that may offer possible benefit but he cannot be certain that the benefit will outweigh the risk. The risk is not that the patient will die but that the undesirable effects of radiation will appear more severe in the terminal cancer patient and that the time of death may be destined to coincide with the undesirable effects of radiation. Supportive treatment, even though

it may not influence the course of disease, must be included in the plan of treatment for such patients. The following case history illustrates some of the problems involved.

A.S. This 73 year old lawyer was admitted with history of hacking, productive cough, left chest pain, fever 102° and generalized lymphadenopathy of 7 weeks duration.

Date	Day	
2/20/63		On admission, examination revealed bilateral cervical, supraclavicular, axillary & inguinal nodes 1 - 3 cm. in diameter; liver 4 cm. and spleen 3 cm. below the costal margin. Chest x-ray - enlarged hilar nodes; lymphangiogram demonstrated enlarged pelvic nodes, bilateral, with filling defects & replacement of normal architecture by tumor. Urine & sputum culture - positive; tetracycline 250 mg. q.i.d. Biopsy - lymphosarcoma, lymphocytic cell type. Bone marrow: diagnosis - diffusely involved by tumor, 275 cc. stored. Hgb. 9.2; WBC 4,500.
3/15/63		Antibiotics continued with little benefit; nodes increasing in size. Hgb. 6.8; WBC 4,100; Plts. 192,000
3/16/63	0	Total body irradiation 185r/6 days, 2 m.v. No nausea or vomiting
3/21/63	5	Hgb. 6.8; WBC 2,500. Transfusion 500 cc. whole blood to combat infection; 50% regression in size of nodes and masses.
4/2/63	17	Hgb. 8.8; WBC 1,100; Plts. 190,000
4/6/63	21	Sputum still positive; Rx colymycin, isolation
4/8/63	23	Re-infusion of marrow not feasible Transfusion - 1000 cc. whole blood
4/12/63	27	Hgb. 11.2; WBC 177; Plts. 28,000
4/14/63	29	Expired.

Comment. Anemia and infection were present on admission and treatment was delayed for nearly a month while an attempt was made to improve the patient's general condition. During this time, nodes and masses continued to enlarge, anemia progressed, and it was evident that there was no chance for improvement unless treatment for lymphosarcoma was offered. Marked regression of nodes indicated good therapeutic response but this was overshadowed by bone marrow depression in the presence of extensive bone marrow involvement and infection. Figure 1 shows response of white blood cell count compared with response of total circulating granulocytes.

Although 68 patients died of disease within 6 months following therapeutic total body irradiation, many of these experienced temporary remission and contributed valuable information to the study. The following case history is an example of good therapeutic effect providing opportunity to observe response through the perio of bone marrow depression and recovery.

J.C. This 37 year old man was admitted complaining of epigastric pain with hematemesis of 5 years duration; jaundice with enlarged liver (7 cm. below costal margin) and retroperitoneal mass (6 x 8 cm.) with widespread lymphadenopathy had been present for 3 months. Biopsy of cervical node revealed lymphosarcoma.

Day	Course
-6	Hgb. 8.8 gm; WBC 4,500 Radiotherapy to abdomen (100r/1 day) 2 m v
-3	Patient very ill, bedridden, requiring heavy narcotics Bone marrow aspiration (320 cc. stored)
0	Total body irradiation 200r/7 days (100r x 2) 2 m v
11	Regression of all nodes and masses, jaundice cleared
28	Hematopoietic depression; Hgb. 6.8; WBC 866; Plts. 14,000 Re-infusion of bone marrow
39	Continued depression; Hgb. 6.8; WBC 431; Plts 54,000
45	Beginning recovery; WBC 2,138; Plts. 90,000
68	Remarkable improvement; patient ambulatory Hgb. 11.2; WBC 3,900; Plts. 212,000
90	Remission continues; WBC 6,200
103	Recurrence of symptoms, developing lymphadenopathy Hgb. 12.1; WBC 2,880; Plts. 130,000
115	Radiotherapy to enlarging abdominal mass (150r/1 day) Moderate relief
151	Hgb. 8.1; WBC 1,710; Plts. 96,000. Expired.

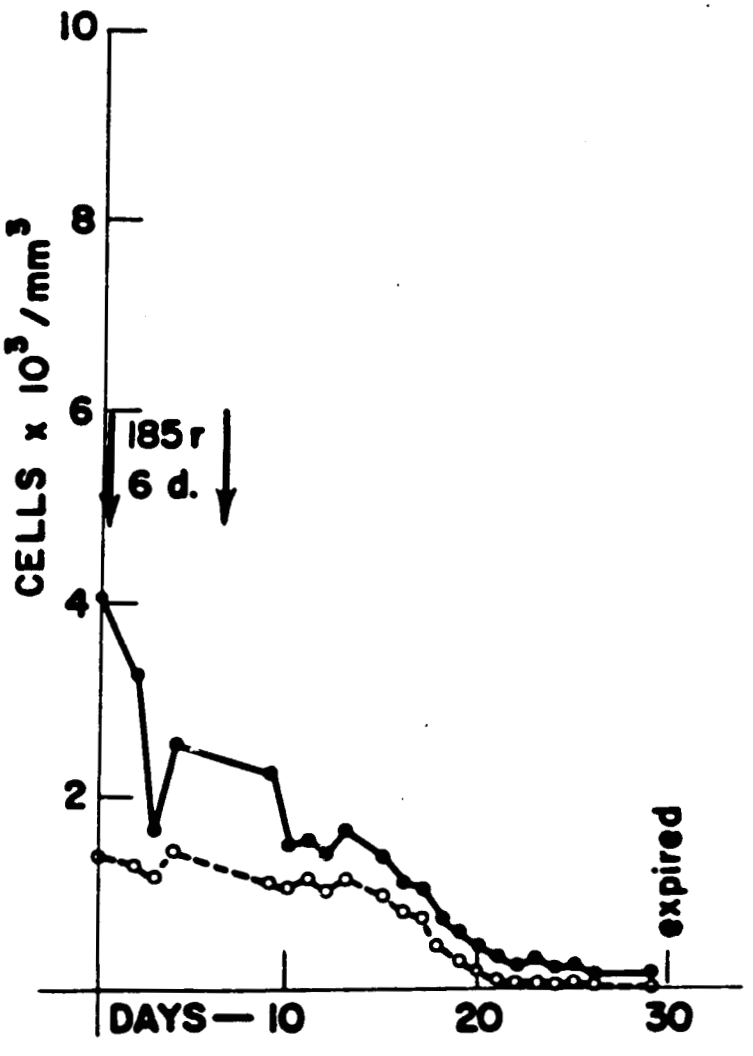
Comment. Survival of 5 months post irradiation permitted observations throughout the depression and recovery period accompanied by remission lasting nearly 3 months and eventual recurrence of symptoms. During this time, multipe hematologic and biochemistry studies were performed. In evaluating effect of radiation, the marked anemia associated with the disease must be considered. Severe anemia was present on admission and, although blood count was normal following recovery from total body irradiation, anemia recurred with exacerbation of symptoms. Thus, total body irradiation was not solely responsible for the severe bone marrow depression following treatment but probably was responsible for improvement in the blood picture occurring 3 months post treatment by virtue of improvement in general condition. Figure 2 shows response of white blood cell count and total circulating granulocytes.

Among humans exposed to irradiation, the development of leukemia (7, 8, 9), sterilization, and changes associated with premature aging have been attributed to latent effect of irradiation, and practically all congenital anomalies are consiered mutations arising from effect of radiation of progenitors (10, 11). While genetic effects require generations to become evident, and the amount of total body irradiation required for sterilization far exceeds the levels used for therapeutic purposes, delayed effects might be observed in patients with prolonged survival.

Total Body Irradiation

Fig. 1.

(A.S.) Comparison of response of w
granulocytes following 185r/



cell count and

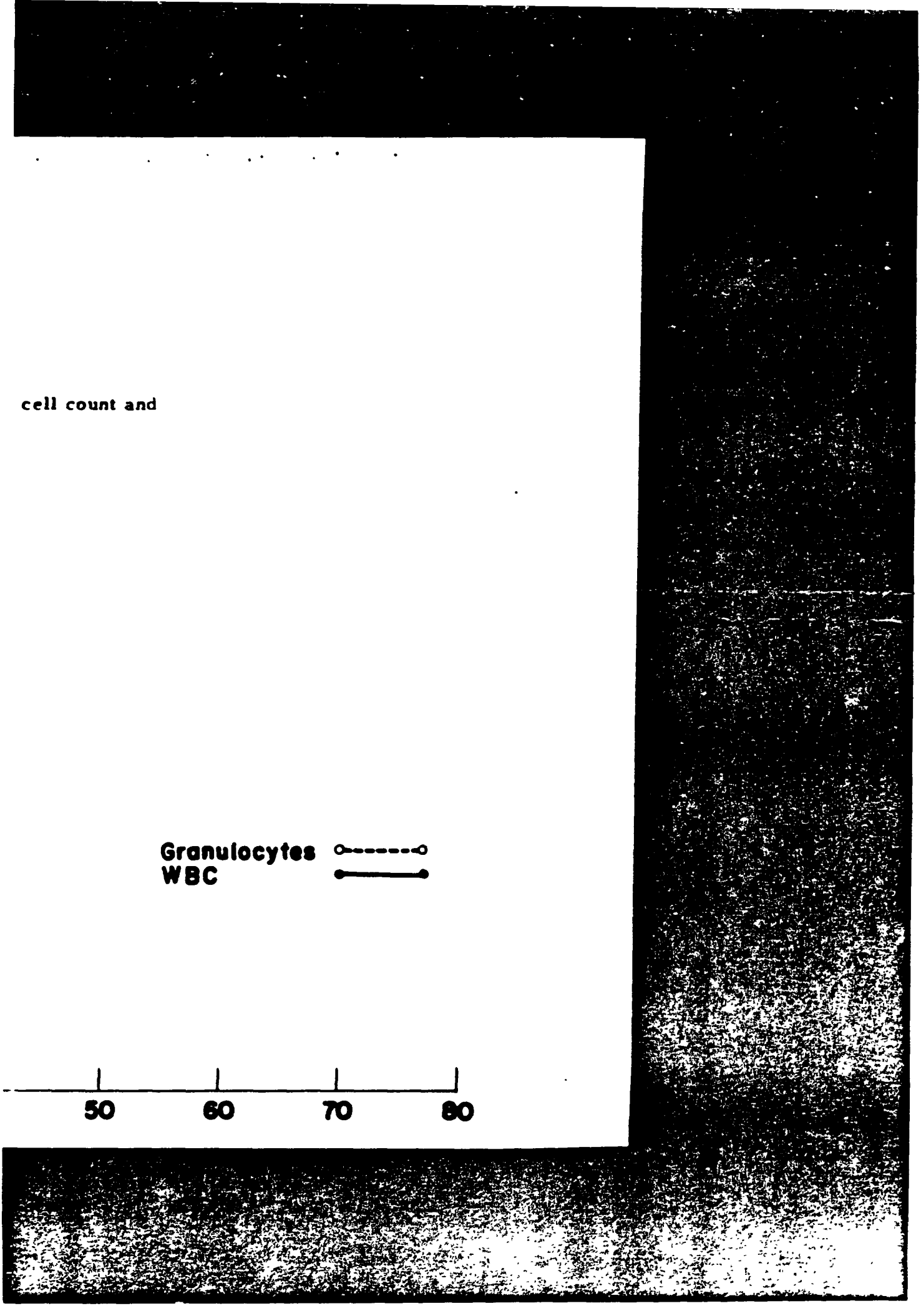
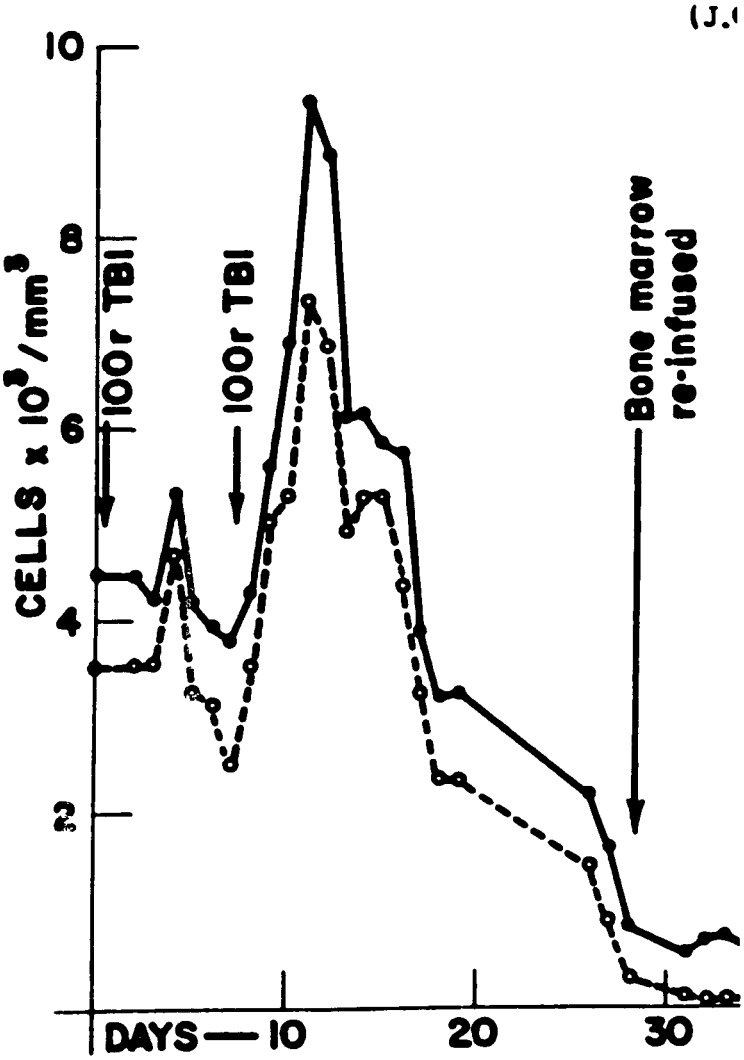


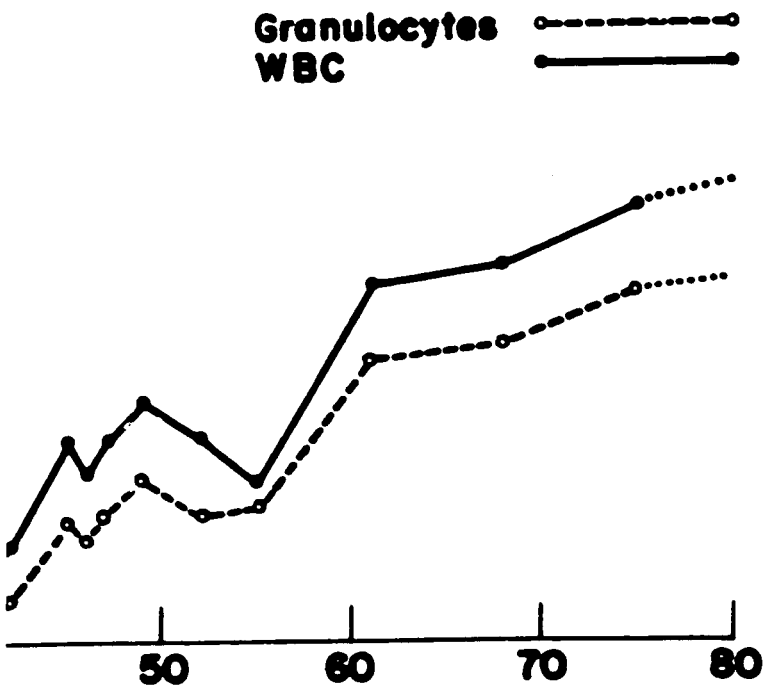
Fig. 2 Total Body Irradiation

Comparison of response of white blood c
granulocytes following 200r/7 days

(J.



and



As with immediate radiation change, the reliability of evaluation of latent effects must depend upon the accuracy of measurement of irradiation, time lapse between exposure and observed change, and measurement of change. In accidental exposure, or exposure associated with occupatient (medical or industrial), the amount of radiation can only be estimated while, in occupational exposure, both amount of radiation and time lapse are difficult to ascertain. In any type of exposure, latent changes due solely to irradiation are influenced by mechanisms of continuous wear, aging and repair. In this series, 9 patients were followed from 6 to 10½ years and were observed for possible late changes. These patients are particularly appropriate for this study because amount of radiation is known and time lapse to last observation has been established. Effect of radiation must be evaluated on the basis of changes that: 1) are known to result from radiation, 2) are not compatible with age or disease, and 3) cannot be accounted for by heredity or chance. Table 9 summarizes amount of radiation exposure and present status with elapsed time for the 9 long-term survivors.

Table 9

Patient	Age	Irradiation	Present Status & F.U. Blood Count
1. J.R.	83	545r/16 days	Died - HCVD 6 yrs. Post Rx. Age 89 Hgb. 13.5, WBC 9,000
2. S.P.	70	125r/1 day	Died - HCVD 7 yrs. Post Rx. Age 77 Hgb. 13.2, WBC 6,400
3. C.W.	64	75r/1 day	Lost - 7 yrs. Post Rx. Age 70 Hgb. 13.0, WBC 6,400
4. B.J.	27	200r/1 day	Lost - 7 yrs. Post Rx. Age 34 On parole from penitentiary, no final WBC
5. G.M.	24	50r/1 day	Living - 7½ yrs. Post Rx. Age 31 Hgb. 12.8, WBC 7,200
6. R.P.	28	25r/4 days	Living - 7½ yrs. Post Rx. Age 35 two children - ages 5 & 3 yrs. Hgb. 15.2, WBC 7,500
7. A.B.	54	50r/1 day	Living - 7½ yrs. Post Rx. Age 61 Hgb. 13.8, WBC 7,500
8. A.H.	60	50r/1 day	Living - 10½ yrs. Post Rx. Age 70 Hgb. 13.0, WBC 6,200
9. I.G.	75	100r/1 day	Living 10½ yrs. Post Rx. Age 86 Feeble, in nursing home Hgb. 10.0, WBC 4,400

The minimum amount of radiation causing specific delayed changes that can be noted clinically in humans cannot be determined; it may exceed the level of therapeutic exposures used, or more time may be required for change to become evident. In this group, no changes were noted that could be attributed to radiation exposure. J.R. (No. 1) received an exposure which, of the entire group, would be most likely to produce latent effect. Since the practice of radiotherapy must balance competing risks of disease with risks of treatment, this patient's age, history of bilateral orchiectomy and generalized disease were weighed against the possibility of radiation change. Tolerance was improved by protraction of radiation and there was excellent therapeutic response. The history of this patient warrants reporting.

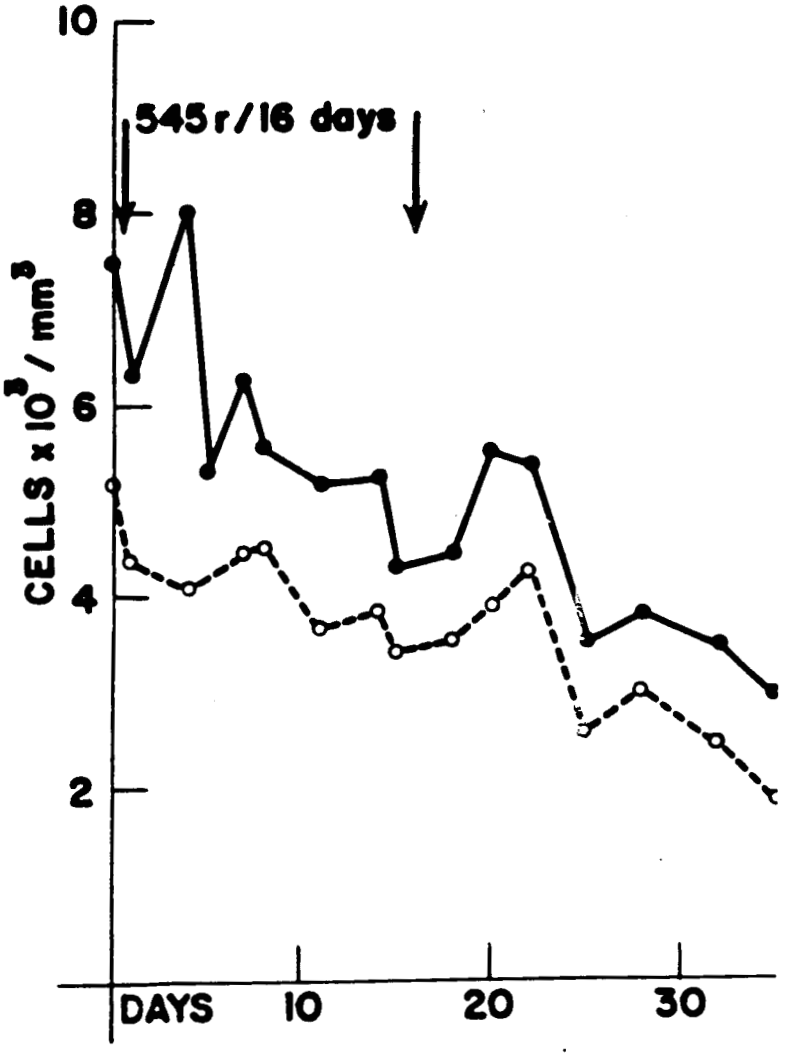
J.R. This 83 year old man was admitted with signs and symptoms of a testicular tumor. Bilateral orchiectomy was carried out and pathology report was lymphoma. Six months later, he developed difficulty in swallowing and examination revealed a firm lesion on posterior tongue with enlarged right mandibular nodes. Biospy again revealed malignant lymphoma.

Day	Course
0	Hgb. 14.1; WBC 8,000; Plts. 196,000 Total body irradiation 545r/16 days Malaise toward end of course with some nausea & vomiting
35	Mass at base of tongue decreased in size; mandibular nodes disappeared. Lowest point of hematopoietic depression Hgb. 12.8; WBC 3,000; Plts. 54,000
67	Recovery well under way. Lesion of tongue disappeared. Hgb. 13.0; WBC 4,000; Plts. 117,900
355	Approx. 1 year post irradiation. Feeling well Hgb. 13.4; WBC 5,500; Plts. 180,000 Normal activities
5 yrs.	No complaints, no evidence of disease Hgb. 12.9; WBC 5,700; Plts. 170,000
2220	6 yrs. + 1 mo. Cerebral accident. Hgb. 13.5; WBC 9,000
2222	Expired.

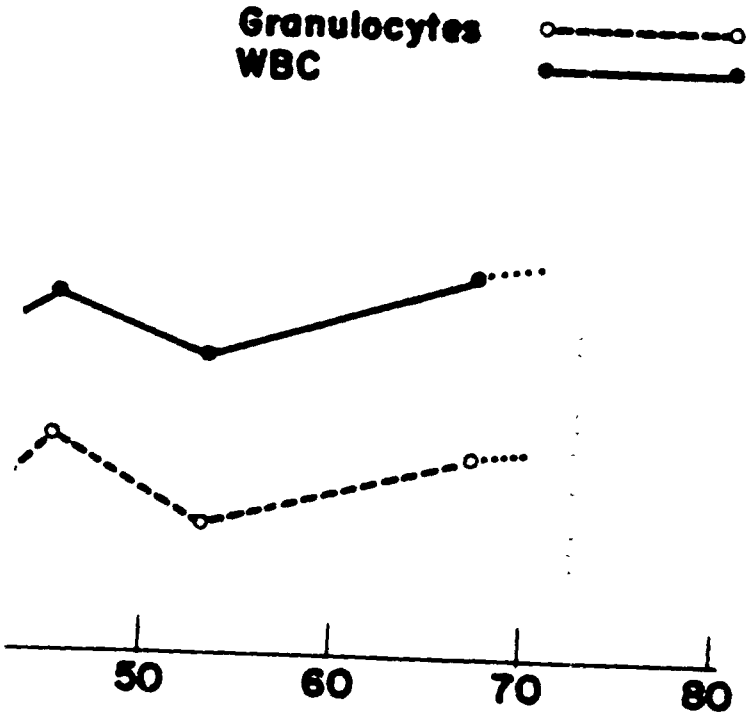
Comment. Because of the likelihood of recurrent disease, this elderly patient was seen frequently for the first 2 or 3 years post irradiation and at 6 month intervals thereafter. Five years following treatment, he was normally active, required glasses only for reading, and had no complaints. Blood count 2 days before death was normal and there was no evidence of radiation change. He died following a cerebral accident at the age of 89 years. Comparison of response of granulocytes with total white blood cell count is shown in figure 3.

Total Body Irradiation

Fig. 3. (J.R.) Comparison of response of whi
granulocytes following 545r/16



ell count and



During the past few years, the protocol of procedures for patients undergoing therapeutic total body irradiation was extended to include some 30 biochemistry tests, and physiology studies including gross body composition and monitoring of vital functions. The protocol required that baseline studies be obtained prior to irradiation with all tests repeated at specific intervals following treatment. No conclusions on this aspect of the work could be drawn because: 1) a large number of patients would be required to provide a standard baseline for cancer patients and to observe changes that might be correlated with amount of radiation, 2) many patients were unable to tolerate multiple tests because of poor general condition, and 3) several patients died before the series could be completed. The complete protocol would have value if it could be applied to all patients receiving therapeutic total body irradiation in several centers throughout the country. This would provide an adequate number of patients for study with opportunity for proper evaluation. However, in the biochemistry studies, results indicate that several tests, namely urea nitrogen, glucose (fasting), serum amylase and serum lipase, merit further investigation.

In body composition studies (described in detail in the Progress Report for period ending 1/31/63), increase in extracellular water and decrease in intracellular water were thought to be due to stress of total body irradiation but changes in lean body weight and fat content could be attributed to weight loss associated with the course of the disease or to total body irradiation. The considerable variation in changes in blood volume led to an intensive study to develop suitable techniques for measuring blood volume and the application of these techniques to changes following other forms of stress.

Monitoring of vital functions with the 6-channel physiograph was carried out on several patients before, during and after irradiation. Constand recording of respiration (rate and depth), blood pressure, electrocardiogram, electroencephalogram and cardiotach failed to reveal definit changes in function except for deceleration of heart rate which coincided with a feeling of nausea in two patients. Both of these patients received partial body irradiation, one to a large abdominal field and one to entire brain. The amount of irradiation to these areas exceeded the level used for therapeutic total body irradiation which may account for the changes noted. In animals, marked changes in vital function were noted following massive radiation exposures (see progress report submitted Jan. 31, 1963).

Animal Experiments

Studies in the latent and genetic effects of total body irradiation have been carried out in animals (usually rats, mice or rabbits) by means of biochemical tests and histologic examination of specific tissues (13, 14, 15, 16, 17). Some investigators have reported both histological and biochemical changes in specific tissues, particularly testis, liver and thymus (see Literature Review). These have contributed little information because

1) experiments produce different results in the hands of different investigators, 2) in some experiments, the amount of radiation used to induce change causes death, preventing long term observations, 3) such changes are the direct effect of radiation and cannot be construed to reflect genetic effects if the animals had survived, 4) techniques of exposure in experimental work are apt to produce disproportionate absorbed dose, i.e., for a given amount of radiation the per cent fall-off in the middle of a small animal would be much less than for a large animal or for a human, and 5) the response of rats, mice and rabbits to irradiation differs considerably from that of man and the findings cannot be applied to humans. For these reasons, it is felt that studies on higher animals might be useful in determining effects that might be expected in humans, and all animals receiving sub-lethal exposures for various purposes throughout the study were retained for observation.

In the course of a study undertaken to investigate the role of bone marrow in recovery from heavy exposures of total body irradiation in dogs, a group of animals have survived from 9 months to 4 years. These animals provide opportunity for long term observations following moderate to heavy exposures ranging from 300r total body irradiation to 1200r total body irradiation with shielding of iliac marrow. In the latter group, four animals received 1000r to the previously protected iliac marrow. Tables 10 and 11 show the amount of irradiation, protection of iliac marrow, course, and survival time.

Table 10

Total Body Irradiation - Experimental Animals (Dogs)
with Shielding of Iliac Marrow

No.	Sex	T.B.I. with shield	Iliac Marrow Irradiation	Survival	
57A	F	1000r	1000r p̄ 9 mos.	3 yrs. 11 mos.	doing well
15A*	F	700r	none	4 yrs.	doing well
13A	F	1200r	1000r p̄ 9 mos.	3 yrs. 11 mos.	doing well
9A	F	600rx2/3 days	none	3 yrs. 4 mos.	doing well
16A	M	1200r with hydrocortisone	none	3 yrs. 3 mos.	paralysis hind quarters
31A	M	1000r	1000r p 14 days	3 yrs. 3 mos.	doing well
14A	M	1000r	none	3 yrs. 1 mo.	doing well
18A	F	1000r	1000r p̄ 14 days	3 yrs.	doing well

*Animal bred 2 months post irradiation with unirradiated male; offspring (not included in table) 62A female, and 63A female, both normal.

Table 11

Total Body Irradiation - Experimental Animals (Dogs) with Bone Marrow Transplants

No.	Sex	T.B.I.	Bone Marrow Transplant	Survival
36B	M	300r	Autologous	9 mos. Doing well
47A	F	300r	none	9 mos. Doing well
35B	M	300r	Isologous	10 mos. Doing well
48A	F	400r	none	11 mos. Doing well

Moderate to severe bone marrow depression developed in all surviving animals but, with recovery, all blood counts returned to normal. Except for one dog (No. 16A), all animals are healthy and frisky; there is no evidence of cataract or other damage to lens, and no evidence of anemia or other blood dyscrasia. No. 16A developed paralysis of hindquarter 2½ years post irradiation. X-rays reveal no abnormality and the animal's blood count is normal; further investigation is being undertaken. In appearance, animals with light-colored coats are unchanged. One black-coated dog is completely gray and the black area of one black and white dog is gray.

One animal, not included in the tables, received 1400r total body irradiation with iliac marrow shielded. This dog recovered but died of distemper 37 days post irradiation. At the time of death, there was no clinical evidence of radiation injury; his white blood cell count was 8,150 and platelets were 70,000. Autopsy showed normal activity of marrow of iliac crest with many mitoses in marrow of long bones.

In this laboratory, proper preparation of animals and studies in dosimetry to deliver an even distribution of radiation have made it possible to establish the LD-100 for dogs at 700r total body irradiation. One animal (15A), receiving 700r with shielding of iliac marrow was bred two months post irradiation. This dog has now survived 4 years. The offspring (62A and 63A) were healthy, normal puppies, developed normally and are now adult animals. If these first generation post irradiation animals could be maintained and bred with other animals surviving moderately heavy exposures with and without shields, and with unirradiated controls, they would represent the origin of possible mutations in future generations. A breeding schedule, designed to demonstrate transferable effects of radiation, has been prepared but it is recognized that such a project is impractical because of the many generations required to produce observable genetic effects.

Apart from the possible origin of genetic effects, continued observation on all surviving animals for latent radiation effects is of considerable

interest. If the amount of radiation exposure received by these animals is sufficient to cause delayed effects, then the development of anemia, leukemia or other malignant process, and acceleration of the aging process (other than graying coat) might be expected. Since the time for delayed effect to become evident varies (7, 8, 9, 13), only continued observation offers the possibility of relating amount of radiation, time and radiation change. A review of reports of animal experiments dealing with total body irradiation by other investigators indicates that no other series receiving near lethal exposures has been maintained long enough to make these observations.

Granulocyte Proliferation Study

The peripheral blood count has always been considered the best index of radiation change but the nature and shape of dose response curves of major classes of cells do not fully explain the role of bone marrow in recovery from total body irradiation. Bone marrow depression is reflected in peripheral blood cells originating in bone marrow, i.e., red blood cells, platelets and granulocytes. The time at which changes in these elements will become evident is a function of the rate of production and the survival time. Granulocytes, having the shortest life span (up to 4 days), are the first to reflect radiation change. Among animals receiving lethal exposures of total body irradiation with a portion of iliac marrow shielded, it was possible to study production of granulocytes by bone marrow examinations and by peripheral blood counts.

The compartments or stages of development of the precursor granulocytic elements found in bone marrow have been described in terms of number of cells and time spent in each compartment (18, 19, 20). Using information obtained from the literature and observations from our own experiments, a model of granulocyte proliferation was constructed for humans and for dogs. The proliferation pathway for each is the same except that time spend in each stage of development in the dog is shorter than that in man.

This is a self-perpetuating system at the stem cell level. As the result of a single division in a 48 hour period, two daughter cells are produced. One retains the characteristics of the parent cells and divides again in 48 hours; the other goes through transformation to a myeloblast. This is followed by successive divisions in promyelocyte and generative myelocyte stages with transformation through the other stages. A single stem cell will release 16 mature segmented neutrophils into circulation every other day. There is another group which releases mature segmented cells into circulation on alternate days. In the steady state, self-perpetuation is the result of self duplication at the stem cellI level. To obtain an increase in stem cells proportional to the rate of recovery following total body irradiation, these cells need only to reduplicate one additional time. The result is 4 stem cells, two return through division the same as the

original parent stem cell of the steady state cycle. The result of this altered stem cell cycle is the creation of a steady state cycle every other day. As each myeloblast results in 8 mature granulocytes in circulation, the ratio of cells being added to the peripheral circulation can be determined.

Utilizing this modification, the recovery rate in terms of circulating granulocytes can be determined with indications to the apparent stage of development in marrow at the time of irradiation. Calculations indicate that the number of cells in each compartment of development surviving the direct effect of radiation can be evaluated quantitatively and correlated with amount of radiation.

In the application of this aspect of granulocyte proliferation to humans receiving therapeutic total body irradiation, a quantitative estimate of cells may be compared to the observed peripheral granulocyte count. In a patient receiving 200r therapeutic total body irradiation, comparison led to the following conclusions.

1. Cells present in peripheral circulation at the time of exposure are not destroyed by this amount of radiation; survival time in circulation is equal to the pre-irradiation value.
2. Cells which are in the transformation compartment continue on to maturigy.
3. All cells in the dividing-transformation stages are lost.
4. Approximately 20% of stem cells in the pre-myeloblast stage are lost; the remainder continue to normal maturity.
5. 50% of parent stem cells are lost; 7 % are altered to form the non-steady state recovery cycle. The remaining 43% continue division according to the steady state cycle but only until the 11th or 12th day. These cells are apparently unable to continue division and are lost.
6. Only those cells produced by the altered cycle are capable of continued division, leading to eventual recovery.

A quantitative analysis of the number of stem cells destroyed by irradiation can be determined by the 14th day post irradiation. Plotting the number of destroyed stem cells against increasing air dose, the resulting sigmoid curve closely resembles the LD values determined for dogs and postulated for humans by Cronkite (21). By extrapolation from estimates obtained by work on experimental animals, effect of higher doses of radiation in humans may be estimated. In both animals and humans, calculations indicate that repopulation of depleted marrow can be accomplished if as little as 6% of stem cells survive. A comparison of granulocyte count with total white blood cell count following total body irradiation is shown in figures 1, 2, and 3.

Studies in granulocyte proliferation have particular application in bone marrow replacement for depression following total body irradiation. If each transplanted stem cell is established in marrow sites on the day of infusion and maintains its ability to divide and progress through the steps of maturation, progeny cells would appear in circulation about the 10th day post infusion. Following single exposures of therapeutic total body

irradiation, the time of greatest depression occurs 25 to 30 days post treatment. Thus, if calculations are correct and bone marrow replacement is an effective treatment, severe bone marrow depression might be avoided if transplantation is carried out prior to the 10th day post irradiation.

Work so far indicates that cells of the granulocyte series may offer advantages as a biologic index of radiation and, by comparison, may permit estimation of amount of irradiation received, as in cases of accidental exposure. The study also provides a basis for estimating: 1) the amount of radiation that would cause death by bone marrow depression, 2) the amount of marrow that must be protected or transplanted to sustain life following exposure and 3) the time when transplanted marrow would be of greatest benefit.

Bone Marrow Bank

Developed first in animals, techniques of aspiration, processing, storage and re-infusion of bone marrow were quickly extended to humans. Since that time there has been no lack of clinical material for bone marrow studies. Marrow is aspirated and stored at -80° C. for all patients receiving therapeutic total body irradiation, for many patients receiving chemotherapy, and it has been recommended that patients in remission from disease have marrow stored against the time when exacerbation of symptoms causes severe blood dyscrasia. Autologous bone marrow has been re-infused in humans, and autologous and homologous marrow has been transplanted in animals.

Samples of marrow are tested for viability at the time of aspiration, at intervals during storage, and prior to re-infusion. In some instances, the amount of marrow obtained was small and the entire amount has been used for repeated viability tests. Among the patients whose marrow is presently in storage, the majority have died but marrow is saved to check decreasing viability with increasing time in storage. Table 13 summarizes the activities and present status of the bone marrow bank.

Table 13

Bone Marrow Bank

Patients		
Marrow aspirated, processed and stored		70
Marrow re-infused	10	
All stored marrow used for viability studies	16	
Marrow now in storage	44	
Animals		
Marrow aspirated, processed and stored		72
Marrow re-infused or transplanted	62	
Marrow in storage	10	

Using standard tests, viability of marrow at the time of re-infusion has been approximately 90% but, clinically, this has been difficult to confirm. Among the patients and animals studied, marrow cells surviving the direct effect of radiation may be responsible for recovery and, except in cases where exposure is of the order of LD-100, survival itself is not proof of viability of re-constituted marrow. Presently, there is no satisfactory way of determining viability and, if viability were certain prior to re-infusion, there are still problems of determining whether viable marrow cells find their way to marrow cavities and proliferate, and whether proliferation is sustained to contribute to recovery of bone marrow depression. Experiments have been designed to overcome these difficulties by in-vivo labeling of marrow cells with tritiated thymidine in experimental animals.

Observations on the effect of total body irradiation in humans and animals have given rise to problems require basic investigation and each year experiments have been conducted to provide the needed information. These have dealt with the role of total body irradiation in suppressing immunologic response to transplants of organs and tissues (32, 24,) and with experiments designed to confirm the work of other investigators. During the past year, an experiment was undertaken to determine lethality in mice following total body irradiation; results are summarized in Table 14.

Table 14

Lethality of Mice Following Total Body Irradiation (220 Kv.)

Exposure	650r	700r	750r	800r
No. of animals	27	30	30	50
LD-50	15 days	10 days	8 days	7 days
LD-65	20½ days	16½ days	9 days	8 days
LD-75	0*	23 days	11½ days	8½ days
LD-100	0*	0**	14 days	13 days

*35% survivals; ** 25% survivals.

The surviving animals have been followed for more than 8 weeks with no further deaths.

Although it is recognized that lethality curves for mice (or rats) cannot be extrapolated to humans, experiments with small animals demonstrating good correlation between amount of radiation and response are deceptively simple. In 1950 Hennessy and Huff reported distinct correlation between radiation exposures ranging from 5r to 25r and erythrocyte depression as determined by iron turnover studies in rats (22). The experiment was repeated in this laboratory, using identical methods, materials and exposures but no correlation between dose and response could be established. In both clinical work and research dealing with total body radiation, experience

indicates that exposures of 5r to 25r are not likely to produce changes in the hematopoietic system that can be detected by any parameter available for measurement.

Literature Review and Bibliography

Because of the apparent gap between basic investigation and clinical observations on total body irradiation, a review of articles and reports dealing with this subject was undertaken. The material, covering a 15 year period (1947 - 1962), necessarily included related publications concerning detection, dosimetry, hazards and protection. Among those dealing with protection, a considerable number were devoted to anti-radiation drugs.

Of more than 600 reports, 78 were selected because: 1) they were representative of all studies concerned with biologic and physiologic response to radiation, 2) results were recorded, 3) results were supported or contradicted by different investigators. In the entire survey, only 10 reports concerned total body irradiation in humans and these are included in the 78 selected. Reports concerning accidental exposure were omitted because amount of exposure was uncertain. Among the 10 concerned with humans, four dealt with continuous low-level exposures of medical and industrial personnel (presumably exposures did not exceed the maximum permissible dose). One (Cronkite - No. 22) discussed clinical response following heavy accidental exposure (i.e., nausea, vomiting, loss of consciousness, etc), three dealt with studies in renal function, one with renal and liver function and one (Nickson - No. 59) emphasized hematology with one of two patients showing electroencephalogram changes following irradiation to the head. Reports on individual cases of accidental exposure were omitted because amount of radiation was uncertain.

Since work in this laboratory on humans has provided ample information on response of the hematopoietic system to irradiation, no effort was made to compile similar data on small animals. No reports were available on the possible application of granulocyte response as a biologic index of radiation exposure.

The 78 reports are listed with name of investigator, reference, amount of irradiation, species and results (pages 21-33) and results have been summarized according to organ or tissue studied, type of test, amount of radiation, species and reference (pages 34-37). A diagram has been prepared showing studies designed to demonstrate changes at the molecular level, biologic effects, metabolic disturbances, morphology, and recovery or death (figure 4).

Among the 68 reports representing many types of gross and histologic examinations and biochemistry studies in animals, in vivo and in vitro, results vary considerable, with frank contradiction in some cases (see increase vs. decrease of weight of testis, Nos. 9, 3, 77, 78; increase vs. decrease in survival with increasing energy Nos. 26 and 31). Variations

may be due to differences in species, size of animals, amount and quality of irradiation, uneven distribution of radiation, and single or protracted exposures. Approximatly one-half of the investigations were carried out on rats and mice while the remainder included rabbits, guinea pigs, ducks chickens, dogs, monkeys and in vitro studies.

Since it is not possible to investigate changes due to moderately heavy radiation exposures in normal humans, investigators must study animals with a view to comparing and extrapolating response to that which might be expected in humans. However, the value of animal experiments for this purpose is questionable when exposures exceed the LD-100 for humans. Following exposures of 10,000r, 30,000r or 750,000r, it seems likely that any looked-for change could be noted and that any stress of comparable severity, such as boiling in oil, would be equally effective. In the maze of incomparable material, comparison of results is not possible and extrapolation of results to that which might be expected in humans would not be meaningful. Yet, the survey has value in pointing out the many approaches to the problem while indicating the areas of need. These areas include:

1. Despite all the data available on hematologic studies, a definite quantitative relationship between amount of radiation and response has not been recognized.
2. Information concerning response to irradiation in humans by means of biochemistry studies is incomplete.
3. There are no reports dealing with physiological studies of total body composition and vital function changes following irradiation.
4. The contribution to the effect of total body irradiation of other stress or injury that might be encountered has not been explored.
5. Results of numerous, independent investigations cannot be evaluated because of diversity of materials, methods and aims.

Effect of Radiatio

	Title and Reference	Or Ar
1.	Albaum, J. Serum enzymes after whole body radiation. Rad. Res. 12:186-194, Feb. 1960	ra 70
2.	Allegretti, et al. Effect of whole body irradiation on Langerhans islets. Rad. Res. 13:31-36, July 1960	gu 25
3.	Allen, J.D. & Jacobson, L.O. Hyper-heparinemia: cause of hemorrhagic syndrome p total body irradiation. Science 105:388, 1947.	Do
4.	Alpen, E.L, et al. Effect of radiation on lymphoid tissue in vivo. Intern. J. Rad. Biol. 2:425-439, 10/60 NSA 1/31/61, p. 169	rat

emistry Studies

Findings

liation

Of 14 enzymes studied, glutamic oxalacetic transaminase was best early indicator of damage. 3 enzymes decreased, 4 were elevated. Sham studies caused changes in 10 of 14 studied.

Histological examination of beta-alpha cell ratio; smallest islets showed greatest increase at 24 hrs. with later shifting to larger islets. Transformation of islet cells can occur.

Thrombocytopenia plays only secondary role.

1. Radiated dog blood delays clotting of normal blood.
2. Antiheparin substances returns clotting time to normal in vivo & in vitro.
3. Isolated heparin-like compound
4. No correlation between hemorrhage & thrombocytopenia
5. Vit. K, ascorbic, Ca. salts & whole fresh blood transfusions did not prevent onset of hemorrhage

DNA synthesis of large & med. lymphocytes were more radiosensitive in vivo than in vitro.

Histological & autoradiographic techniques permit separation of effects due to population changes from those due to biochemical alteration. Among controls, average generation time for large & med. lymphocytes of lymph nodes is 20-30 hrs. Evidence is in favor of a longer life span for the small lymphocytes.

	Title and Reference	Effect of Radiation Organi: & Dose
5.	Anderson, D.R. Effect of radiation on creatine metabolism. Rad. Res. 1957, p. 300	1500r rats
6.	Barron, E.S., et al. Mechanism of action of ionizing radiation. I. Inhibition of enzymes. J. Gen. Physiology 32:537-552, 1949	in vitro 25 - 50
7.	Ibid. Idem. 32:595-605, 1949 II. Inhibition of sulhydral enzymes	
8.	Bettendorf, G. Berlin. Strahlenterapic, Urbon & Schwargenberg 112:74-78, 5/60 Effect of total body radiation in intermediate produces of nuclei acid metabolism.	rats 800r
9.	Boling, J.L. & Floyd, R.L. Biological effects of high intensity microsecond pulses of x-irradiation as determined by post-irradiation testicular weight loss. DA-49-007-901, Report 3/60. TAB 2/1/62, p. 104.	mice
10.	Bond, Fliedner & Cronkite J. Nuc. Med. Oct. 1960	
11.	Botkin, et al. Thyroid response to total body irradiation. Endocrinology 50:550, 1952	rats 1000r

iistry Studies

Findings

Marked creatinuria (20 x normal). 3 - 4 fold increase in plasma creatine. No change in urine creatinine or or guanido-acetic acid.

Inhibition of phosphoglyceraldehyde dehydrogenase 50% after 200r, 100% after 500r. Hexokinase - little change. ATP at pHG.1 inhibited at 10r with 100% at 1000r. Succinoxidase inhibited proportional to concentration. No inhibition of enzymes without sulphydral groups.
Inhibition was dependent on type of radiation (alpha, beta, gamma) with some effects at 14r. Maximum occurred by the 6th day.

Changes in activity & concentration studied by labeled phosphate. Those showing decrease in thymus by 30 min. were: ADP, GTP, TTP, DPN + changes at 7 hrs. which were: AMP, ATP, CTP, UTP. Decreased activity shown by P^{32} uptake by 30 min. in TTP, AMP; by 7 hrs. in ATP, CTP, GTP, UTP, & RNA.

Testicular weight loss doe to irradiation at high dose rate is roughly comparable with that reported due to relatively low dose rate.

Results summarizedseparately

ncrease in thyroid activity after 2 hrs. apparent for 24 hrs. Normal by 6th day.

	Title and Reference	O &
12.	Bowers, J.Z. & Scott, K.G. Distribution & excretion of electrolytes p acute total body irradiation injury. Studies in radio-sodium Proc. Soc. Exp. Biol. & Med. 78, 1951	7
13.	Bowers et al. Effect of acute whole body irradiation on salt & water metabolism & significance. Radiology 61:97, 1953.	ra 66
14.	Brent, R.L. et al. Effect of radiation on serum transaminase level. Rad. Res. 1957, p. 305	70 ra
15.	Breyer, F.T. et al. Effect of radiation on adreanl cortical steroid excretion in urine. Science 120:112, 1954.	pi 7 1
16.	Brown, C.S. et al. Biochemical, cellular & bacteriologic changes in thoracic duct lymph p total body irradiation. Am. J. Physiol. 163; 668, 1950	do 50
17.	Brown, S.O. & Krise, G.M. Chronic whole body radiation stress. Ann. Prog. Report 2/61. Radiation Biol. Lab. College Station	r 0, d
18.	Carter, W. Variation in tissue DNA p total body irradiation. Fed. Proc. 12:188, 1953	r L

chemistry Studies

Findings

Edema when histopathological findings were pronounced. Occurred when radio Na & K were decreasing. Decrease in urinary excretion of radiosodium corresponds with blood decrease and increased G.I. injury.

Decreased food & water intake. Negative balance of Na, K, Cl for 1st 3 days. Loss of K & gain of Na in small intestine at 24 hrs. Increase of K in liver @ 24 hrs.

Increase in glutamic acid oxalacetic transaminase within 24 hrs. No correlation to pre-irradiation values or death.

Marked increase in excretion of adrenocortical steroids within 1st 24 hrs.

Decrease in T. protein within 50 hrs. Increase in NPN in 5 hrs. Decrease in keratin values by 30% by 17 hrs. Sl. change in sugar. No change in Cl. Lymphs decreased to 18% by 18th hr. with low of 13% by 132 hrs. These results slightly different from blood studies done at same time. Bacterial cultures negative on blood & lymph.

e Decrease - WBC, platlets, erythroid elements @ 10r/day
r No change in glucose homeostasis. Decrease in tolerance to therman stress.

:s Tissues: muscle, skin, gut, liver, kidney, spleen & testis
Analysis: DNA, H_2O, fat, Na, K, Cl. Marked increase of DNA in all tissues except kidney during 1st 4 hrs.
In rats - decrease in intra- extracellular fluids

Effect of Radiation -

	Title & Reference	Organi & dose
19.	Cavalieri, R.R. et al. US Naval Hosp. Decreased urinary excretion of 2-aminoehtanol by irradiated humans. NSA 4129 Nature 191:1303-1304, 9/23/61	huma 300r-
20.	Conrad, R.A., Cronkite, E.P. et al. 3/56 Experimental Rx of G.I. tract syndrome produced by lethal doses. Naval Med. Research Inst. Bethesda	Dogs lethal
21.	Constant, M.A. & Phillips, P.H. Effect of multiple low-dose radiation & splenectomy on stability of dog erythrocytes. Am. J. Physiol. 178:367, 1954.	dogs 100
22.	Cronkite, E.P., & Bone, V.P. Diagnosis of radiation injury & analysis of human lethal dose of radiation. Armed Forces Med. J. March 1960	huma dose-
23.	Detrick, L.E. et al. Thiamine transport across irradiated rat intestine Rad. Res. 15:520-526, Oct. 1961	rats 525r
24.	Dicksen, E.A. & Shapiro, B. Effect of gamma radiation on viscosity & enzymatic activity of urease solution. Rept. No.61-85, Sept. 1961, TAB 2/1/61, p. 101	in vit Co-6 750,
25.	Dorfinon, R.J., Rosenfeld, G. Steroid excretion in calf urine. 1/58 Naval Med. Res. Inst. Bethesda	calf

istry Studies

Findings

Among normal controls & cancer patients, urinary excretion of aminoethanol, although variable, was never less than 10 mg./day; p whole body irradiation, aminoethanol decreased to undetectable concentrations (less than 0/4μ gm/ml., reappears by the 12th day.

Anorexia, vomiting, diarrhea, fluid & electrolytes loss and vascular collapse.

Marked decrease in fragility when compared to non-splenectomized, irradiated dogs. Both were lower than normal (less fragility). Rise noted in several in terminal stage. No correlation to life expectancy, of Hgb. levels, platelets or target cells.

Human data collected from all accidental exposures see summary (No. 10)

Following 525r there is decreased absorption of thiamine probably related to subcellular damage in crypt cells in rats.

Specific viscosity of urease decreases initially, then levels off at about 3/4 the original value. Protection against irradiation produces viscosity in urease solutions containing $(GES)_2$ which inhibits enzymatic activity, probably due to intramolecular rearrangement

Urine 17-keto. & 17-hydorx. - increase utilization of I.V. hydrocortisone

	Title and Reference	Organi & dose
26.	Ellinger, F. et al. Lethal effect of 200 & 2000 Kv. x-rays & Co-60 gamma rays. Rad. Res. 1957, p. 313.	guinea 325-75(
27.	Esnouf, M.P. et al. Biochemical studies 6 - 18 mos. post TBI. J. Radiat. Biol. 3:459-466, 9/61	mice
28.	Ibid, idem p. 467-473, 9/61	mice
29.	Fransen, Fr. Ann. Tech. Rept. Cologne, U. Germany. Formation & pathophysiological significance of biogenic amines in sublethal radiation injuries.	rats 500r
30.	Geisselroder, J. & Nims, F.F. Carbohydrate metabolism in stress. Rad. Res. 1957, p. 317	rats
31.	Goldfeder, A. & Clark, G. Evaluation of "Time Factor" in radiobiological effects Rad. Res. 1957, p. 318.	mice 700r
32.	Graham, Douglas & Sacher. Chronic radiation mortality p single T.B.I. Rad. Res. 1957, p. 319.	mice

try Studies

Findings

LD-50 dose increased by 1/3 to ½ between 200 Kv, 2000 Kv and Co-60. Definite dependencey on type of radiation & exposure geometry on mortality.

There was reduction in the incorporation of P^{32} into kidney DNA relative to that in RNA

Irradiation produced permanent depression of DNA synethesis relative to RNA in the kidneys

5-hydroxytyramine - results not reported
Noradrenaline & histamine - results not reported
5-hydroxytryptamine - urine increase 6th-10th day
5-hydroxindol - increase 2 x in urine
Ethylamine & isoamylamine - no increase
Allylamine & vinylamine - both increased

Blood sugar, liver glycogen and muscle glycogen showed increases

100r @ 8.6r/min, 25% survival
100r @ 17.2r/min. 47.8% survival
100r @ 830r/min, 66.6% survival. No significant weight loss of whole body, spleen, liver or testis.

Increasing LD-50 with decreasing energy (250 Kv-634r; 35 Kv-663r; 80 Kv-816r) no sex differences, no leukemia, tumors or degenerative disease differential for all qualities. Same relative effect.

	Title & Reference	Organism & dose
33.	Hanford, S.W. Acute radiation syndrome p TBI in supralethal dose 8/19/60, Vol. 18 (report) Naval Med. Res. Inst. Bethesda	dogs LD-100 Co-60
34.	Hale, W.M. & Stones, R.D. Effect of ionizing radiation on immunity. Radiation Res. 1:459-469, 1954	
35.	Hawrylewicz, E.J. Effect of TBI on blood enzymes. 6/30/60 Armour Research Foundation, Chi.	rats 0.3-300 for 26 w
36.	Ingram, M., et al. Occurrence of lympho-cytes with bi-lobed nuclei in cyclotron personnel. Science 116:706, 1952.	human low lev continu
37.	Jackson, K.L. et al. Electrolyte excretion following severe intestinal damagy by x-irradiation. Naval Radiolog. Defense Lab. San Francisco 6/57	rats 1500r
38.	Jacobson & Krohn, D.O. RBE of neutrons by correlating physiologic change of eye Cont. AF33(616)712, proj.7165 TAB 2/1/62, p. 105	rabbits

ry Studies

lndings

,B.C. - decrease, all elements.
Ser. Prot. & electrolytes - no changes
lood vol. - significant decrease at pre-mortal period
epatic blood flow & renal blood flow well maintained
ıtil immediate premortal stage.

ata from various investigators: inhibitory effect on
ıtibody response is intimately related to dose, species,
ne of stimulus, soluble or particulate nature of antigen,
ot of stimulus. Radiation reduces or abolishes active
· passive immunity to bacterial & animal parasite in-
ction. Little or no effect on acquired immunity to
ral infections or bacterial toxin.

)-enzyme A, aldolase, Glu-6-phosphate dehy., Glu.
tol. Trans, DPN - all increased with 300r/d - 300r/wk.
ıtalase LDH, carbonic anhydrase, cocarboxylase,
ıvin adomine dinucleotide - none showed any specific
ange.

:rease in lymphs with bilobed nuclei
:turned to normal after protection

% increase in K excretion in urine over 4 day interval
8% increase in sodium (in G.I. tract)

e damage based on electroretinogram, biochemical
:erminations, histopathological & clinical observations
24 hrs. post exposure. RBE varied with different
licators but appeared to be not less than 1 & not greater
n 3. Higher energies appeared to have much greater
E.

	Title and Reference	Organi & dos
39.	Kaufman, J. The coagulogram as a critical indicator of irradiation effect. Am. J. Roentgenol. 55:464, 1946	hums low I
40.	King, E.R. Use of total body irradiation in Rx. of far advanced malignancies JAMA 9:10, 9/2/60	hums 300r
41.	Kinsey, V.E. Effect of neutrons & other radiations on ocular lens. Contr. AT(11-1) 152. Kresge Eye Inst. Detroit NSA 12/15/61 p. 3925	ani
42.	Klein, J.R. Brookhaven. Protoporphyrin & heme formation by erythrocytes from irradiation & bled duck Am. J. Physiol. 210:663, 10/7/61 NSA p.4128	ducl 800-
43.	Klouwen, H.M. Radiosensitivity of nuclear RNA. Biochemica et Biophysics Acta 12:366-368, 8/12/60	rat 700
44.	Kohn, H.I. Changes in blood plasma during acute radiation syndrome. Am. J. Physiol. 162:703, 1950	rat 200
45.	Krise, G.M., et al. Radiation creatinuria School of Aviation Med. Randolph AFB Oct. 1957	rats neutr gamn
46.	Idem, Ibid May 1957	750r 1500r

itry Studies

Findings

Platelets, clot retraction, bleeding time, fibrin time, complete coagulation time & protrhombin time altered fairly consistently.

No increase of taurine urinary excretion until 300r or over. B-aminoisobutyric acid increases. Fibrin deposit in lungs after large single doses.

Study concerns the transport of amino acids across the blood aqueous barriers, the capsul & epithelium of the lens by relative concentration of amino acids in aqueous & vitreous humors, lens & plasma. No results so far.

In vitro studies show an increase in pro heme & free protoporphyrin formation by erythrocytes p frequent bleeding. Decrease in heme formation but not in free prophyrin production was observed.

In separated nuclei from irradiated liver & thymus cells, RNA was labeled with P^{32}; liver cells showed slight increase while thymus cells showed marked decrease in uptake.

Glucose increased 60 mg.% 1st day, normal 5th day. NPH elevated for 5 days; A/G ratio rose from 3.1 to 4.5 1st 2 days, normal 5th day, due to nonprotein fractions. Decreased total protein 5th-7th day. Elevation chloride & cholesterol 3rd-5th day.

p mixed irradiation, no change p 5th day in urine creatine; with gamma only, creatinine decreased and creatine increased.

1500r - 20 to 30 x creatinuria on 2nd day; 435r mixed - 36 to 63 x creatinuria on 2nd day; profound creatinuria (85 x) at death.

	Title and Reference	Organi & dose
47.	Kawade, M. Nippon. Effect of x-rays on the affinity of liver for protoporphyrin III. NSA 8/31/61, p. 2666	guinea 100r T
48.	Ibid, idem	local 1000r ht 2000r gt pi
49.	Ibid, idem Re: renal function	Guinea 50r-8,0
50.	Lamberts, H.B. An immediate radio-biological effect which is simple to record. Medica Mundi.6:44-47,1960	cock's c rat & ht skin
51.	E.H. Labrosse & Smith, J.E. Urinary coproporphyrin excretion p gamma irradiation. School of Aviation Med. Randolph AFB 2/56	rats, 10 250r, 50 750r & 1000r
52.	Leone, C.A. Effect of irradiation as revealed by serological analysis. Tr. Kan.Acad. Sci.60:301-315, 1957	chickens 330-990
53.	Lott,J.R. & Wilding,J.L. Attempt to demonstrate lethal toxin in blood p irradiation. Sch. Aviation Med. Randolph AFB, 6/56	rats 1000r - 20,000r

ıtry Studies	
	Findings
light change in parenchymal cells with some decrease affinity for protoporphyrin, but after 14 days, metabolism returned to normal. p 400r-1000r, there was generation of parenchymal cells but no change in terstitial tissue & epithelial cells of bile ducts.	
00r hepatic radiation strongly impaired dye excretory nction of liver by BSP test. Administration of B_2, 12, or glucuronic acid each day p exposure - disrbance was reduced. BSP in pts. receiving 1000r over per abdomen showed similar results.	
nal function decreased p exposure. Regardless of dose, antity of urine was decreased; P^{32} in bladder was creased in accordance with dose while P^{32} in blood & nal area was increased.	
anges in macromolecular mucopolysaccharides. There s immediate reaction giving a drop in viscosity of novia as measured by pressure of injection.	
dose relationship with coproporphyrin in urine	
lr - no changes; 660r depressed activity at 24 hrs, mal p 3 days; at 30-60 days, serological activity eeded that of controls. Major changes occur in the lobulins of the plasma	
toxin demonstrated on gross transfusion	

	Title and Reference	O; &
54.	Luzzio, J. Electrophoretic patterns c x-irradiated blood serums. Army Med. Res. Lab. Fort Knox. Report AMRL Proj. 6 X 64-14-001-01	in
55.	McGoodall & Long. Effect of whole body radiation on adrenal medulla & on adrenalin & noradrenalin. Am. J. Physiol.197:1265-1270, 1959	ra ra 80 30
56.	Milch, L.J. et al. Plasma lipoproteins & muscle adenosonetriphosphate in rabbits p local radiation. Sch. Aviation Med. Randolph AFB. Dec. 1955	ra 30 hi
57.	Mole, R.H. Whole body irradiation & the idea of stress. Brit. J. Exper. Path.37:528-531, 1956	ra 20
58.	Nickson, JJ. & Koch, H.J. Postirradiation syndrome in humans. Coproporphyrin excretion, serum protein levels. Report #3 AFSWP-743, 3/31/55	hu
59.	Ibid, idem. Dec. 1959	hu 25 88 1
60.	Parizek, J. Excretion of desoxycytidine in urine p irradiation. "The Cell Nucleus" N.Y. Academic Press, N.Y. 1960, p. 82-88. NSA 12/15/61, p. 3918	ra

:hemistry Studies

Findings

Electrophoretic changes produced in vitro irradiation of serum with massive doses parallel those changes observed after in-vivo exposure to lethal and sub-lethal exposures.

Little change in noradrenalin titre p 800r but immediate decrease in adrenalin which returned to normal by 64th day; at 1600r decrease to zero; return of synthesis noted by 72 hrs. Rabbits & cats supported rat study.

Varying dose rates. Increase in plasma lipids, decrease in lipoprotein, independent of dose. No change in muscle ATP

Release of cell contents from destroyed cells is responsible for the increase in blood sugar p irradiation, not increased adrenal activity. Increased liver glycogen is not due to increased adrenal activity.

Results not reported

Detailed biochem. studies; hematology gave most consistent results. Of 2 pts. receiving irradiation to head, + eeg., 1 recorded subclinical explosive phenomena at 4 and 6 hrs.

Irradiation increase the excretion of desoxycytidine. Amount correlates well with amount of radiation even at relatively low dose levels (dose not given).

Effect of Radiatio

	Title and Reference	Orga & dos
61.	Pitcock, J.A. Late testicular lesions in irradiated monkeys (path. & clinical studies) Report # 61-72. TAB 2/1/62, p. 101	mo 57 154
62.	Roth, L.J. Irradiation effects on C.N.S. Contract AT(11-)-847 NSA 3925, 12/15/61	rats 10,00
63.	Sandberg, A. & Doull, J. Influence of low level gamma & fast neutron irradiation. I. Effect of aging & irradiation on adaptation to decreased barometirc pressure & glucose tolerance. A.F. Radiation Lab. Chicago. NSA 12/15/61, p. 3924	mice
64.	Seston, N., et al. Effect of sublethal TBI on glucose tolerance. Rad. Res. 13:25-30, 1960	rats guine 250r,
65.	Shiels, D.O. Structural aspects of lympho-cytes & monocytes relative to clinical condition p irradiation. Brit. Med. J. 32:306.	huma low l
66.	Skalka, M. (Czech) Transaminase level in blood plasma p single & repeated doses. NSA 12/15/61, p. 3927.	mice
67.	Slenson, J.R. Effect of TBI on some con-constituents of liver & kidney. Proc. Soc.Exper. Biol.&M. 82:707 1952.	rats 880r 1000r

·mistry Studies

Findings

Testicular lesions were found 3 - 8 years p exposure to gamma radiation. Degree of damage was independent of dose rate & fractionation bu is dependent primarily on the cumulative dose.

No significant change in permeability properties of the blood brain barrier was detected for the human serum albumin or sulfate in irradiated brain.

Aged mice exhibit impaired glucose utilization, and irradiation exposure in the sublethal range decreases ability of both young & old animals to eliminate excess blood glucose.

Greatest impairment of glucose utilization on 8th day Believed to be extra-pancreatic in origin.

Increase in large lymphs with decrease in lymphs showing granules. Increase in monocytes of a particular type. Significant changes in ratios of these cells related to exposure.

Increase in glutamic-oxalacetic transaminase (GOT) & glutamic-pyruvic transaminase (GPT) in plasma. Max. increase @ 8 hrs., a second peak may occur @ 7 days. Further increase when irradiation was repeated p 4 days but repeat exposures after 7 or 10 days produced no increase.

Decrease in ATP in liver 1st day, rising on 4th day. No change in phosphate distribution in kidney. Fasting p exposure - decreased rate of glycogenolysis. Increased sulphydral content in liver. Little change in cytochrome oxidase content.

	Title and Reference	Orgs & de
68.	Smith, E.D. et al. Physiological & biochem. studies p massive irradiation Rad. Res. 11:198-205, 1959.	450- cont mic bats frog
69.	Soeda, H. Effect of irradiation on liver function in pts. with malignant tumors. Nippon. NSA 8/31/61, p. 2667	86 h loca
70.	Spoerl, E. & Quiner, W. Glucose uptake & dissimilation by irradiated, starved & division inhibited yeast. Report # 486 Army Med. Res. Lab. Fort Knox	yeas
71.	Staukovic, F. Effect of TBI in insulin resistance of fasting rats. Nature 184: Suppl.32, 1816, 12/59	rats 600r
72.	Stocken, L.A. Observations on biochemical effect of radiation. Rad. Res. Suppl. 1, 53-57, 1959 (summary of reports by different investigators)	

nistry Studies

Findings

All died after 100,000r! Immediate decrease in
its, rectal temperature in homotherms. No dignificant
rs alterations in enzymes.
l,
ls

Tests: Kunkel zinc sulfate, sublimate turbidity, BSP bilirubenia, S. cholinestorase. Exposure over abdomen - unfavorable reaction on liver function.

A concurrent lower rate of glucose uptake of un-irradiated, starved cells as compared to irradiated cells. Discussion - possibility that membrane is site of cellular change involved.

6 of 20 rats survived insulin shock compared to 20 of 20 controls. Decrease in hexokinase activity was also observed.

nzymes

ATP - Doubrise & Peterson - ATP increase in mouse spleen proportional to dose between 25 - 400r. Similar findings in thymus and for 5-nucleotidase

Catalase - in vivo & invitro with occasional reduction in activity

Nucleases - two DNAases are now recognized, DNA I & II. 500r - increase in DNA II in liver. No change in DNA I.

xidation of Phosphorylation

Mitochondria - Potter & Bethell - Reduced P/O ratios in spleen mitochondria taken from rats exposed to 800r one hr. previously.

Van Bekkum - Succinate as substrate, reduced P/O ratio after 100r in spleen & 50r in thymus. At 700r the earliest significant depression in spleen at 2 hrs. No effect in liver at 2 or 4 hrs; nuclear degeneration at 1 hr. with reduced mitotic index at 15 min.

Effect of Radiation

Title and Reference

72. Stocken, L.A. continued
cont'd.

III. C
A.

B.

C.

IV.

mistry Studies

Findings

of Phosphorylation

ggie - Oxidation of various members of Kreb's cycle
er mitochondria. 50r inhibited pyruvate oxidation by
rate by 10 - 15%. 100r before effect on succinate.
60% inhibition of oxygen uptake 30 to 60 min.
Phosphorylation - Creasey & Stocken. Only intra-
iosphorylating systems can separate radiosensitive and
tive tissues. 100r or more suppressed phosphorylation
thumus, bone marrow, lymph node nuclei.
ation of acid soluble phosphates - levels of ATP and
eotides seem to depend on both the tissue and time after
. Rat thymus - no change in P^{32} specific activity,
ion of inorganic phosphate or ATP @ 1 hr. post 1000r.
Mann - Rabbit marrow, marked fall in ATP concentration
- no change at 4 hrs. after 700r. At 24 hrs. or 3 days
reduced concentrations in rat liver, rat & mouse spleen
. The cytochrome C effect may reflect damage to the
ria with a consequent reduction in phosphorylation.
id. Comprehensive review: 1. Hevesy & Forsberg,
Internat. Congr. Biochem., Brussels 1955, 479, 1956.
G. Brit. J. Radiol. 29:465, 1956. 3. Evera, M.
der Protoplasmaforschung (L.V. Heilbrunn & F. Weber,
41). Springer, W. 1957. 4. Kelly, L.D. Prog. in Bio-
em. 8:144, 1957. Reduced amounts of DNA & RNA have
ved in certain tissues 3 hrs. post irradiation, but changes
kely due to disappearance of cells.
: A. Howard - Ciba Foundation Symposium, ionizing
cell metabolism 196, 1956: "Most experimental results
ained as due simply to mitotic delay and cell death
require to invoke a biochemical action of radiation
thesis per se."

	Title and Reference	Organ & dose
73.	Ueno, I., et al. Eosinophilia developed during x-ray therapy. Vestnik Rentgenol.I Radiol.35:55-58, 1960	human 250-41 6000-9
74.	U. Nuvolone. Italy. Renal function in irradiated patients NSA 8/31/61, p. NSA p. 2667	human 2000r partial
75.	Vogel, H.H., Frigerio, N.R. & Jordan, D.L. Life shorteningin mice p fission neutrons or Co-60 gamma rays at low dose rate	mice
76.	Weiner, N. et al. Time trend of hyper-lipoproteinemia p radiation injury. Sch. Aviation Med. Randolph AFB 1955	rabbits local
77.	Ibid, idem. Effect of reduced food intake on hyperlipoproteinemia of local radiation injury.	rabbits
78.	Yamamoto, G. Effect of radiation on nuclei acid metabolism. I. Radiosensitivity of various organs. NASA 8/31/61, p. 2666	mice 200-300r

stry Studies

Findings

ocal exposures. Tendency to either an absolute or elative increase in eosinophil count.

maximum tubular excretion of paminohippuric acid PAH) is a particularly sensitive & useful measure enal failure during the early post irradiation period. ages have been noted p 2000r when part of the field aded the renal area.

dose rate is much less effective in shortening span than is high dose rate.

d plasma lipids 1st week, persisted for several weeks. otein curves. Muscle histology & ATP levels show sion of local tissue changes after radiation.

pidemia, weight loss.
i controls developed increased plasma lipid & ein with little weight loss.

ucleic acid content of thymus, spleen, liver & testis easured. Liver is the most radio-resistant with other showing decrease in wt. & nucleic acid content. acid metabolism was disturbed in thymus, spleen & y small sublethal exposures.

Effect of Radiati

Summ

Tissue, organ or system	Observations and Changes
Urine	Decreased urease activity
Kidney	Coproporphyrin (no report)
	" no dose relatio
	Increased deoxycytidine
	Creatine - 20% increase
	17 keto - 17 hydroxy increased
	Increased adrenal cortical ster
	Increased beta-amine isobutyri
	Increased chlorine excretion
	No increase in taurine
	Decreased aminoethanol
Intestine	Decreased thiamine absorption
Eye	increased damage
Synovia	Decreased viscosity & cohesiver
Blood cells	Depression - hematopoietic sys
	" " "
	Increased RBC, Hgb, WBC c shi
	Increased L/S lymphs, L+M/s s
	Decreased granulocytes, increa
	Decreased DNA synthesis of lyn
	Decreased lymphs
	Increased amines
	Decreased RBC fragility
	Increased lymphs with bilobed n
Brain	No alteration in enzyme activity
	No change in permeability

iemistry Studies

:sults

	Exposure	Organism	Reference
	750,000r	in vitro	24
		humans	58
	100-1000r	rats	51
		rats	60
	1500r	dogs	45,46
		calf	25
	750-1000r	pigs, rats	5, 15, 37
	300-500r	humans	40
	750-1000r	pigs, rats	5, 15, 37
	300-500r	humans	40
	300-500r	humans	40
	525r	rats	19
		rabbits	38, 41
in		cockscomb, rat, & human skin	50
	300-500r	humans	40
	0-20r/day	ducks & mice	17,33
	low level	humans	73
pe	" "	"	65
rtes	" "	"	65
		rats	4
	500r	dogs	16
	500r	rats	29
	100r/wk	dogs	21
	low level	humans	36
	450-500r	several species	68
	10,000r	rats	62

Effect of Radiation

Summa

Tissue, organ or system	Observations and Changes
Whole body	No weight loss
	Loss of consciousness
	Early nausea
	No vomiting
	Diarrhea in 24 hrs. - poor prognos
	Decreased food intake
	Survivalsincrease c increasing ene
	" " c increasing dos
	" " c decreasing ene
	" " c decreasing dos
Spleen	No weight loss
	Decreased DNA, H_2O, fat, Na, K, C
Liver	No weight loss
	Protoporphyrin (decreased function)
	Decreased ATP, increased on 4th d
	Retarded glycogenolysis
	Increased sulfhydral compounds
	No change in Na, p, H_2O
	Slight increase in cytochrome oxida
	Increased P^{32} uptake in RNA
	Decreased function (several tests)
	Increased glycogen (6 to 10 times)
	Decreased DNA, water, fat Na, K, C
	Oxidation & phosphorylation
Testis	No weight loss
	Wt. loss, same with high & low dose
	Increased incidence of testicular les

ıistry Studies

ults

Exposure	Organism	Reference
700r	mice	31
heavy	humans	22
"	"	22
up to 100r	"	22
heavy	"	22
660r	rats	13
LD-50	guinea pigs	26
700r	mice	31
634-816r	mice	32
80-250 Kv.	rabbits	75
700r	mice	31
LD-50	rats, rabbits	58
700r	mice	31
100r	guinea pigs	47, 48
1000r	rats	66,67
880r	rats	67
880r	rats	67
880r	rats	67
880r	rats	67
700r	rats	43
	humans	69
200r	rats	57
LD-50	rats, rabbits	59
(see summary)		72
700r	rats, rabbits	31
	mice	9
57-154 rep	monkeys	61

Effect of Radiati

Summ

Tissue, organ or system	Observations and Changes
Plasma	No lethal toxin identified Increased clotting time, clot re bleeding time, fibrin time Isolation of heparin-like compo Increased sugar (10 fold) at 24 h Impaired glucose utilization Increased transaminase at 24 h Decreased balance of Na, K, Cl Shift in lipoproteins correlated Decrease beta globulin Decreased antibody formation & c little effect on acquired imm Increased enzymes activity Increased NPN, A/G, Cl, cholest Creatine - 3 to 4 fold increase Protein - (massive, lethal, & sub changes comparable)
Lymph of thoracic duct	Decreased lymphs by 18%, T. P Increased amines, NPN, uric ac
Kidney	No changes in phosphate No change in sulfhydral, Na, por Decreased P^{32} excretion Decreased DNA, H_2O, fat, Na, K Decreased renal excretion of PA
Pancreas	Shift in beta-alpha cell ratio

Bibliography

1. Collins, V.P., Loeffler, R.K. and Hyman, G.A. Comparative effects of total body radiation, nitrogen mustard and triethylene melamine on the hematopoietic system of terminal cancer patients. Science 118:161-163, 1953.

2. Collins, V.P. and Loeffler, R.K. The therapeutic use of single doses of total body radiation. Am. J. Roentgenol. 75:542-547, 1956.

3. Collins, V.P. A study of the effect of total and partial body radiation on iron metabolism and hematopoiesis. Progress Report 1/31/56. AFSWP Contract No. DA-49-007-MD-428.

4. Collins, V.P. The effect of total body irradiation on immunologic tolerance of bone marrow and homografts of other living tissue. Progress Report 1/31/62. DASA Contract No. DA-49-146-XZ-032.

5. Ellinger, F.P. Newer concepts of radiation sickness and its treatment. Proc. Rudolg Virchow M. Soc. 14:9-24, 1955.

6. Sherman, J.D. Etiology of anemia with cancer. J. Lab. & Clin. Med. 60:598-605, 1962.

7. Cooper, A.G. and Steinbeck, A.W. Leukemia following irradiation for ankylosing spondylitis. Brit. J. Radiol. 32:266-268, 1959.

8. Court Brown, W.M. Leukemia and aplastic anemia in patients irradiated for ankylosing spondylitis. Med. Res. Council (Brit.) Spec. Report. Series No. 295 (H.M. Stationery Office, London, 1957).

9. Lewis, E.B. Lewis, F.B. Leukemia, multiple myeloma and aplastic anemia in American radiologists. Science 142:1492-1493, 1963.

10. Research in the Effects and Influences of the Nuclear Bomb Test Explosion. II. Program of ABCC & US-Japan Relationship. Japan Soc. for Promotion of Science, Ueno, Tokyo, 1956. p. 1449-1454, 1495-1500, 1522-1824.

11. The Effects of Nuclear Weapons. Department of the Army Pamphlet No. 39-3, April 1962.

12. Simpson, C.L., Hempelmann, L.H. and Fuller, L.M. Neoplasia in children treated with x-ray in infancey for thymic enlargement. Radiology 64:840-845, 1955.

13. Klouwen, H.M. Radiosensitivity of Nuclear RNA. Biochemica & Biophysics Acta 12:366-368, 1960.

14. Bowling, J.L & Floyd, R.L. Biological effects of high intensity micro second pulses of x-irradiation as determined by post irradiation testicular weight loss. Res. Report DA-49-007-MD-901, April 1960.

15. Vogel, H.H., Frigerio, N.R and Jordan, D.L. Life shortening in mice irradiated with either fission neutrons or Co-60 gamma rays at low dose rate. Argonne Nat. Lab. 6368, Dec. 1961.

16. Yamamoto, G. Effects of radiation on nucleic acid metabolism. I. Radiosensitivity of various organs. NASA Res. Report 8/61, p. 2666.

17. Carter, W. Variations in tissue DNA after total body irradiation. Fed. Proc. 12:188, 1953.

18. Lajthan, L.F. On DNA labeling in the study of the dynamics of bone marrow cell populations. The kinetics of cellular proliferation. Grune & Stratton, N.Y. 1959. p. 173-187.

19. Bond, V.P., Fliedner, T.M., Cronkite, E.P. Rubini, J.R. & Robertson, J. Cell turnover in blood and blood forming tissues studied with tritiated thymidine. Ibid p. 188-200.

20. Patt, H.M. and Maloney, M.A. Kinetics of neutrophil balance. Ibid. p. 201-207.

21. Cronkite, E.P. Diagnosis of radiation injury and analysis of human lethal dose of radiation. U.S. Armed Forces M.J. 2:249-260, 1960.

22. Hennessy, T.G. and Huff, R.L. Depression of tracer iron uptake curve in rat erythrocytes following total body x-irradiation. Proc. Soc. Expertl. Biol. & Med. 73, 436, 1950.

23. Jordan, G.L. Jr., Collins, V.P., et al. The treatment of irradiation sickness in the dog with autologous and homologous bone marrow. Surgery 113; 591-597, 1961.

24. Jordan, G.L., Collins, V.P. et al. Splenic transplantation in the dog. (in press). Presented at N.Y. Acad. Sciences, Feb. 1964.

istry Studies

ults

Exposure	Organism	Reference
700r	mice	31
heavy	humans	22
"	"	22
up to 100r	"	22
heavy	"	22
660r	rats	13
LD-50	guinea pigs	26
700r	mice	31
634-816r	mice	32
80-250 Kv.	rabbits	75
700r	mice	31
LD-50	rats, rabbits	58
700r	mice	31
100r	guinea pigs	47, 48
1000r	rats	66,67
880r	rats	67
880r	rats	67
880r	rats	67
880r	rats	67
700r	rats	43
	humans	69
200r	rats	57
LD-50	rats, rabbits	59
(see summary)		72
700r	rats, rabbits	31
	mice	9
57-154 rep	monkeys	61

Effect of Radiati

Summ

Tissue, organ or system	Observations and Changes
Plasma	No lethal toxin identified Increased clotting time, clot re bleeding time, fibrin time Isolation of heparin-like compo Increased sugar (10 fold) at 24 h Impaired glucose utilization Increased transaminase at 24 h Decreased balance of Na, K, Cl Shift in lipoproteins correlated Decrease beta globulin Decreased antibody formation & c little effect on acquired imm Increased enzymes activity Increased NPN, A/G, Cl, cholest Creatine - 3 to 4 fold increase Protein - (massive, lethal, & sub changes comparable)
Lymph of thoracic duct	Decreased lymphs by 18%, T. P Increased amines, NPN, uric ac
Kidney	No changes in phosphate No change in sulfhydral, Na, por Decreased P^{32} excretion Decreased DNA, H_2O, fat, Na, K Decreased renal excretion of PA
Pancreas	Shift in beta-alpha cell ratio

iemistry Studies

·sults

	Exposure	Organism	Reference
	1000-20,000r	rats	53
me,			
	low levels	humans	39
		dogs	3
	200r	rats, yeast	30,44,57,70
	250r, 500r	guinea pigs, rats	34
	700r	rabbits	1,14,66
	660r	rats	13
	835r	rabbits	77,56,76
	330-990r	chickens	52
	summary of	data	64
	0.3-300r/wk	rats	35
	200r	rats	44
	1500r	rats	5
		in vitro	54
ratin	500r	rats & dogs	16, 29
	1000r	rats	67
	880r	rats	67
	50-8000r	guinea pigs	48
	LD-50	rats, rabbits	58, 27, 28
	2000r		
	partial	humans	74
	250r	guinea pigs	2

Effect of Radiation

Summ

Tissue, organ or system	Observations and Changes
Thymus	Decreased ADP, GTP, TTP, DPN
	" AMP, ATP, CTP, UTP @
	Decreased uptake P^{32}, TTP, AMI
	" ATP, CTP, GTP, UDP @
	Decreased uptake P^{32} in RNA
	Increased ATP
	No change in inorganic P @ 1 hr.
Adrenal glands	No change in noradrenalin up to 6 decreased to 0 by 76 hrs.
	Decreased adrenalin
	" "
	No change in either
Thyroid	Increased thyroid activity

mistry Studies

sults

Exposure	Organism	Reference
. +		
300r	rats	8, 78
n.+		
800r	rats	8
25-400r	mice	72
1000r	rats	72
800r	rats	55, 71
1770r	rabbits	55,71
1600r	rats	55,71
30,000r	cats	55,71
1,000r	rats	11

Figure 4.

EFFECT OF IONIZING RADIATION ON LIVING TISSUE

Summary of Investigations

Disturbance at the Molecular Level
1. Sulfhydryl compounds
2. Splitting of H_2O to form dioxides, peroxides, etc.

Inorganic Radicals (short lived)

Organic Radicals

Damage to Biological Structure
R.B.C. Fragility
Th amine Absorption

Preprophase Arrest
1. (H^3) thymidine
2. (P^{32}) incorporation
3. (Fe^{59}) uptake
4. Cholinesterase

Cell Division
Mitotic index

Metabolic Disturbances

I. Enzymes
- Compound F
- Transaminase
- RNAase
- ATPase
- DNAase
- Catalase
- S-cholinesterase

II. Amino acids, Amines, Nucleic acids
- B-aminobutyric acid
- Creatine, creatinine
- Biogenic amines
- 2-amino ethanol
- Deoxycytidine
- RNA & DNA
- Bilirubenia

III. Mineral metabolism
- Sodium
- Potassium
- Chlorides
- Iron
- Iodine

IV. Proteins
- 1. Nitrogen
- A G ratio
- Lipoproteins
- Mucopolysaccharieds
- B-globulins
- Immunochemistry
- Protoporphrin metabolism

V. Steroids
- 17 keto - 17 hydroxy

VI. Others
- Carbohydrate metabolism
- Glycogen
- Glucose
- Cholesterol
- Renal function
- Kunkel zinc sulfate
- Sublimate turbidity
- B-P
- etc.

Genetic unbalance

Mutation
Chromosome abberation

Morphological Change
Cataract
Vascular collapse

Partial or complete recovery
Bone marrow depression
G.I. tract injury

Sterilization

Differentiation
1. Ratio of cell types
2. Rebuck test

Death
1. Single cell type - hematopoietic system, testis, intestinal mucosa
2. Organ - systemic effects, necrosis, RISA absorption, etc.
3. Organism - survival time, increased aging

It is possible that the areas of need (outlined on page 20) may be fulfilled by means of an over-all, coordinated program with all participants using a common, comprehensive protocol of procedure. Such a protocol would include dosimetry studies to achieve uniform distribution of irradiation, hematology, biochemistry, and physiology tests, and observations on subjective response. With the accumulation of sufficient material, all data might be evaluated by computer methods to assure reliability of analysis.

If a well coordinated program failed to provide the needed information, then it seems likely that only relative quantitation is possible. While prediction of effect of radiation in a small volume of tissue is possible, prediction becomes more difficult as volume of tissue increases. Measurement of change and function of complex biologic systems presents many problems even under normal conditions so it is not surprising that effect of total body irradiation, which involves all tissues, organs and systems undergoing constant changes of wear and repair, would present inexplicable problems in measurement. If effect of total body irradiation is non-specific because of the many factors involved, then evaluation of the organism as a whole may provide information overlooked in the search for a specific biologic index. Evaluation of the whole individual would relate amount of total body irradiation with performance, degree of incapacitation and survival. Based on clinical observations that take into account biologic and physiologic responses, it would permit prediction of effect of exposures ranging to the LD-100. Such a prediction was presented as an addendum to the progress report of January 31, 1963. This prediction may not be the final answer but, because it represents the "best" answer that can be given from an appraisal of presently available data, it is included in its entirety in this final report.

A PREDICTION OF THE EFFECT OF TOTAL BODY IRRADIATION BASED ON CLINICAL OBSERVATIONS

The essential problem posed by the possibility of massive irradiation of the human is to predict the lethal dose, the dose at which immediate but temporary incapacity will occur, and the dose at which delayed but significant injury will occur. The possible answers proliferate as probable qualifying factors are introduced, e.g.,

1. Unless there is a uniform concept of dose and dosimetric procedures, gross discrepancies in estimates of dose-effect are to be expected.
2. The effects of irradiation are not specific and are apt to be confused with other types of injury,--mental, physical or biochemical.
3. The effect of the accidental or catastrophic irradiation exposure will vary with the geographic, geometric and anatomic relations that may be unsuspected or, at least, difficult to reconstruct.
4. Age, general health and competing disease will modify the response of the individual. Prediction of dose-effect is possible for individuals or for uniform groups of individuals but a very wide rante of dose-effects is to be expected in the general population.
5. Prediction of dose-effect in humans must be based upon the controlled circumstances of clinical use of radiotherapy where uniformity of radiation throughout the body may be sought although this is unlikely ever to be achieved in accidental or catastrophic exposure.
6. Morbidity and mortality will clearly depend upon medical assistance whether this be merely supportive or actually specific.

Nevertheless, some simple expression of the hazard of radiation is necessary and a prediction can be offered if an allowance of $\pm$ 3 d B is granted.

The following predictions are based on a hypothetical group of adult males between 30 and 50 years of age, with training or education to permit a basic understanding of radiation and biology, physically and mentally conditioned to work in the field of radiation hazards and free of any previous physical or chemical trauma likely to impair tolerance to radiation. The dose is expressed as the amount of radiation measured in air at the site where the individual is exposed. The geometry of exposure and quality of irradiation is assumed to be such as to produce a uniform distribution through the entire body.

"Acute incapacity" means uncertain ability to carry out crucial mental or physical tasks.

"Partial disability" means ability to carry out mental tasks and sedentary work but vulnerable to stress, e.g., severe physical exertion, infection or further irradiation.

The problems of single brief exposure and of protracted exposures are dealt with separately.

Table 15

Predicted Effect

of

Single Exposures of Total Body Irradiation

Amount of Irradiation	Acute Incapacitation	Partial Disability	Blood Counts (% of normal)	Blood Counts Time to Recovery
50r	0	0		
100r	0	0	75%	60 days
200r	0	0	50%	60 days
300r	1 - 6 hrs. (minor)	0	25%	60 days
500r (no deaths anticipated below this level)	1 - 12 hrs.	2 - 3 mos.	5%	90 days
700r (LD-50 range)	1 - 72 hrs.	6 mos.	1%	120 days
900r (LD-100 range)	2 mos.	12 mos. (if any survivors)	<1%	1 year (if any survivors)

The prediction of the effects of protracted total body irradiation is less readily described in simple numerical values for dose and time.

The rule to be anticipate is that, taking values for single exposures of the order of one hour as a standard, the effect of protraction will be (A) to obscure the time of onset of signs or symptoms due to delayed and insidious development , and (B) to prolong the recovery period due to: 1) increased time required for accumulation of an effective amount of irradiation, 2) inhibition of the repairative response, 3) vulnerability to infection and other forms of stress during the period when repairative response is impaired.

It is evident that at some low level of protracted exposure, no detectable effect will occur and at some high level of protraction falling short of lethality, the above description will be clearly applicable. Intermediate exposures will be difficult to define with precision.

Table 16

Predicted Effect
of
Protracted Total Body Irradiation

Amount of Radiation and Duration of Exposure	Symptoms: Acute Incapacitation	Symptoms: Partial Disability	Blood Counts: % of normal	Blood Counts: Recovery time
100r in 1 wk.	0	0	90%	
in 1 mo.	0	0	90%	
in 1 yr.	0	0	0	
200r in 1 wk.	0	0	75%	
in 1 mo.	0	0	50%	
in 1 yr.	0	0	0	
300r in 1 wk.	0	Day 15-50	50%	3 mos.
in 1 mo.	0	Day 30-90	25%	4 mos.
in 1 yr.	0	0	0	
500r in 1 wk.	Day 7-10	Day 10-90	20%	4 mos.
(MLD range) in 1 mo.	Day 25-35	Day 35-150	1%	6 mos.
in 1 yr.	0	Mos. 10-24	50%	4 mos.
700r in 1 wk.	Day 5-30	Day 30-150	1%	6 mos.
(LD-50 range) in 1 mo.	Day 15-50	Day 50-200	<1%	12 mos.
in 1 yr.	0	Mos. 10-24	25%	4 mos.
900r in 1 wk.	Day 4-30	Mos. 1-6	1%	1 yr.
(LD-90 range) in 1 mo.	Day 10-50	Mos. 2-12	<1%	2 yrs.
in 1 yr.	0	Mos. 8-24	10%	1 yr.

The above values are predictions, not conclusions.

They are offered as "best answers" to current conjecture based on total experience in clinical radio herapy.

The values below 300r are firm estimates.

The values below 500r are safe estimates.

The reason for continued clinical investigation is to accumulate further observations and develop more reliable tests to confirm or alter the impressions in high dose ranges.

Bibliography

1. Collins, V.P., Loeffler, R.K. and Hyman, G.A. Comparative effects of total body radiation, nitrogen mustard and triethylene melamine on the hematopoietic system of terminal cancer patients. Science 118:161-163, 1953.

2. Collins, V.P. and Loeffler, R.K. The therapeutic use of single doses of total body radiation. Am. J. Roentgenol. 75:542-547, 1956.

3. Collins, V.P. A study of the effect of total and partial body radiation on iron metabolism and hematopoiesis. Progress Report 1/31/56. AFSWP Contract No. DA-49-007-MD-428.

4. Collins, V.P. The effect of total body irradiation on immunologic tolerance of bone marrow and homografts of other living tissue. Progress Report 1/31/62. DASA Contract No. DA-49-146-XZ-032.

5. Ellinger, F.P. Newer concepts of radiation sickness and its treatment. Proc. Rudolg Virchow M. Soc. 14:9-24, 1955.

6. Sherman, J.D. Etiology of anemia with cancer. J. Lab. & Clin. Med. 60:598-605, 1962.

7. Cooper, A.G. and Steinbeck, A.W. Leukemia following irradiation for ankylosing spondylitis. Brit. J. Radiol. 32:266-268, 1959.

8. Court Brown, W.M. Leukemia and aplastic anemia in patients irradiated for ankylosing spondylitis. Med. Res. Council (Brit.) Spec. Report. Series No. 295 (H.M. Stationery Office, London, 1957).

9. Lewis, E.B. Lewis, F.B. Leukemia, multiple myeloma and aplastic anemia in American radiologists. Science 142:1492-1493, 1963.

10. Research in the Effects and Influences of the Nuclear Bomb Test Explosion. II. Program of ABCC & US-Japan Relationship. Japan Soc. for Promotion of Science, Ueno, Tokyo, 1956. p. 1449-1454, 1495-1500, 1522-1824.

11. The Effects of Nuclear Weapons. Department of the Army Pamphlet No. 39-3, April 1962.

12. Simpson, C.L., Hempelmann, L.H. and Fuller, L.M. Neoplasia in children treated with x-ray in infancey for thymic enlargement. Radiology 64:840-845, 1955.

13. Klouwen, H.M. Radiosensitivity of Nuclear RNA. Biochemica & Biophysics Acta 12:366-368, 1960.

14. Bowling, J.L & Floyd, R.L. Biological effects of high intensity micro second pulses of x-irradiation as determined by post irradiation testicular weight loss. Res. Report DA-49-007-MD-901, April 1960.

15. Vogel, H.H., Frigerio, N.R and Jordan, D.L. Life shortening in mice irradiated with either fission neutrons or Co-60 gamma rays at low dose rate. Argonne Nat. Lab. 6368, Dec. 1961.

16. Yamamoto, G. Effects of radiation on nucleic acid metabolism. I. Radiosensitivity of various organs. NASA Res. Report 8/61, p. 2666.

17. Carter, W. Variations in tissue DNA after total body irradiation. Fed. Proc. 12:188, 1953.

18. Lajthan, L.F. On DNA labeling in the study of the dynamics of bone marrow cell populations. The kinetics of cellular proliferation. Grune & Stratton, N.Y. 1959. p. 173-187.

19. Bond, V.P., Fliedner, T.M., Cronkite, E.P. Rubini, J.R. & Robertson, J. Cell turnover in blood and blood forming tissues studied with tritiated thymidine. Ibid p. 188-200.

20. Patt, H.M. and Maloney, M.A. Kinetics of neutrophil balance. Ibid. p. 201-207.

21. Cronkite, E.P. Diagnosis of radiation injury and analysis of human lethal dose of radiation. U.S. Armed Forces M.J. 2:249-260, 1960.

22. Hennessy, T.G. and Hutf, R.L. Depression of tracer iron uptake curve in rat erythrocytes following total body x-irradiation. Proc. Soc. Experti. Biol. & Med. 73, 436, 1950.

23. Jordan, G.L. Jr., Collins, V.P., et al. The treatment of irradiation sickness in the dog with autologous and homologous bone marrow. Surgery 113; 591-597, 1961.

24. Jordan, G.L., Collins, V.P. et al. Splenic transplantation in the dog. (in press). Presented at N.Y. Acad. Sciences, Feb. 1964.